Practical Plant Anatomy

By

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To my wife

PREFACE TO THE SECOND EDITION

Although the present volume represents a complete revision of the subject matter and laboratory procedures presented in the first edition, its essential purpose remains the same, viz.: to provide for the student a means of articulating the practical study of laboratory material with the best of modern theory and interpretation. During the past seven years, notable advances have been made in our knowledge of many aspects of both comparative and developmental anatomy. Such progress is reflected, in the present book, by the more extended treatment accorded to meristems, sclerenchyma, the structure and ontogeny of the primary vascular tissues, and the development and anatomy of the stem, leaf and root. A new exercise, dealing with the structure and ontogeny of laticiferous tubes, has been added; it is hoped that the laboratory material suggested for this topic will enable teachers to introduce this important but frequently neglected phase of histology into the program of laboratory instruction. As in the first edition, the present volume is largely confined to the study of the "vegetative body" of the seed plants. New material however, both theoretical as well as practical, has been incorporated on the topic of floral histogenesis. This will direct the students' attention to the need for histogenetic investigation in any study of floral morphology. Despite its interest, the anatomy of the flower (and of its products, i.e., fruits and seeds) cannot be included in a book of this type without sacrificing much of the space accorded to the fundamental aspects of the histology of leaf, stem and root. In the authors' opinion, the voluminous data on the comparative anatomy of the flower (especially vascular anatomy) represent subject matter more adapted for advanced work in anatomy and morphology, and hence not within the necessarily limited scope of the present book.

Dr. Katherine Esau has read and criticized the entire manuscript and has also contributed the section on the laboratory study of laticiferous tubes. For her generous co-operation and

valued counsel the author extends his thanks. Appreciation is also due Mr. Ernest Gifford who has assisted the author in many ways in the preparation of this new edition. For all errors in the present book, however, the author assumes full responsibility.

A. S. F.

Berkeley, California
August, 1948

PREFACE TO THE FIRST EDITION

Since a realistic foundation in plant anatomy depends upon thorough laboratory practice, there appears to be a definite need for a guide that will both direct as well as orient the student in his individual studies. The present book has been written from this standpoint and is therefore intended for use in the laboratory. Each exercise contains an introductory section in which an effort is made to summarize briefly but clearly the present status of knowledge of the subject for study. This "Introduction" is in no sense to be regarded as a substitute for collateral reading in the standard texts in plant anatomy and in the selected modern literature which are appended at the end of each exercise. But the author's experience has led to the conviction that a wholly unnatural and artificial gap may easily occur between "theory" and "practice" in the teaching of plant anatomy. To quote from De Bary's classic of 1884, "On the anatomy of plants such an indescribable amount has been written that, in a comprehensive treatise, one or many authors might be cited in reference to every word." The truth of this statement, of course, is self-evident today, and the beginner in anatomy is often confused as well as discouraged by the wealth of detail and maze of controversy presented in many anatomical texts. In the present book, therefore, the aim has been to articulate as far as possible the practical study of laboratory material with the best of modern interpretation and theory. By this means the student, through his own work in the laboratory, should be able gradually to acquire a practical basis for the critical evaluation of theory.

The material suggested for study under each exercise has been selected, as far as possible, from types of plants readily available to most teachers. An effort has been made to avoid rare or unusual plants and frequent reference is made to forms of economic importance to man. Wherever it seemed desirable, alternative material has been listed.

In view of the existence of several excellent texts in plant microtechnique, special methods for the preparation of macerated tissue and permanent mounts, as well as the use of microchemical

reagents, receive only brief attention in this book. However, a few notes on these topics, which may prove valuable to both the teacher and student in the use of this book, are included under the "Appendix."

Since teaching methods vary, especially with respect to the nature of the record that the student is required to make of his laboratory work, each exercise contains a list of suggested drawings and special topical reports. This, it is hoped, will permit of selection on the part of the teacher in accordance with the time and emphasis placed on a given topic.

Whatever practical merits the present volume may possess are due to a large degree to the constructive criticisms of numerous students who used the book in its previous planographed form. The exercise on sieve-tube elements has been read and criticized by Dr. Katherine Esau and Dr. A. S. Crafts for whose assistance the author expresses his thanks. I am also grateful for the many helpful suggestions made by Dr. Ernest Ball who served as my laboratory assistant for the past three years. For all errors in fact or interpretation, however, the writer assumes full responsibility.

A. S. F.

Berkeley, California
October, 1941

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General References

The books listed below constitute the most important general references in plant anatomy and are cited by the author, wherever useful, in the specific reference lists at the end of each exercise. Additional references including recent papers and comprehensive review articles will be found at the end of all of the exercises.

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Exercise I

THE PROTOPLAST

It is now a well-established fact that protoplasm, which constitutes the physical basis of life, is organized and subdivided in the higher plants into small units termed *protoplasts*. Usually each protoplast consists of a mass of cytoplasm and a single nucleus and, in most cases, is invested by a distinct cell wall. Exceptions to the typical condition are furnished by the multinucleate protoplasts of certain fibers (cf. Exercise IX) and laticiferous tubes (cf. Exercise XII). Such *coenocytes* offer problems of considerable phylogenetic and ontogenetic interest. The so-called "Cell Theory," proposed over a century ago, regards the organism both ontogenetically and phylogenetically as a "cell republic" that has arisen by "the aggregation of a vast number of elementary individuals" or cells (Sharp 1934, pp. 20-24). A somewhat contrasted viewpoint, the "Organismal Theory," attaches less importance to the septate condition and regards cellular structure as the *result* of the growth of the organism as a whole. In modern plant histology and cytology, *both* of these apparently antithetic viewpoints have value, for the following reasons. On the one hand, many aspects of ontogeny, e.g., the highly individualistic development of idioblasts (Foster 1944, 1945, 1947; Scott 1941, p. 242), the origin of vessels and articulated laticiferous tubes by cell fusion (cf. Exercise X and XII), and the processes of mitosis, meiosis and cytokinesis, can be most clearly *described* in terms of the cell theory. On the other hand, it seems clear that the differentiation of the varied cell types and tissues of the plant should be *interpreted* as far as possible as *integrated aspects* of the development of the organism as a whole. Sharp (1943, Ch. II), who has admirably reviewed the problems just outlined, states with reference to differentiation: "Cellular structure is accessory, and cell division is an incident of growth rather than the cause of differentiation." Doubtless the definitiveness of the cell wall as a boundary between the protoplasts in

plant tissues has been responsible for the continued acceptance of the "Cell Theory" as the more useful concept, at least in the analytical study of plant histology.

I. The Use of the Term "Cell"

Robert Hooke in 1665 applied the term "cell" to each of the small "cavities" observed by him in various plant tissues. Although Hooke was unable, of course, to observe protoplasmic structure, he was aware, as Matzke (1943) has recently pointed out, that many cells had contents in the form of juices or liquids. Later, with the discovery of protoplasm, the major emphasis was placed upon the structure and behavior of the protoplast and the cell wall was regarded as a "lifeless" excretion of the living substance. At present, however, it seems both convenient and justifiable to include *both the protoplast as well as its wall under the general concept of cell.* Evidence that the protoplast together with its wall represent a biological unit (at least in higher plants) is provided (1) by the intimate relationship existing between the cell wall and the protoplast during cell differentiation and (2) the widespread occurrence of protoplasmic connections or *plasmodesmata* within the primary walls of tissue elements (cf. Exercise II, pp. 14-15. Many important cell types in plants such as sclereids, fibers, tracheids, vessel elements and cork consist only of the cell wall at maturity. However, it is entirely appropriate to designate them as "cells" since the loss of their protoplasts occurs during the final stages of their differentiation.

II. Material for the Study of the Protoplast

1. *Plant hairs.* The cells of many plant hairs furnish very useful material for a study of the protoplast. Because such cells are usually highly vacuolated and hence semitransparent, they may be easily studied without special preparation or staining. Indeed, in the staminal hairs of *Tradescantia,* the brilliantly colored cell sap in the vacuome provides a splendid optical contrast for the gray cytoplasm and nucleus.

Obtain a transverse section near the tip of the stem of petunia or squash, mount it [1] carefully in water and examine the prepara-

[1] For technique of sectioning and maintaining fresh material cf. Appendix pp. 213-214.

tion under *low magnification.* A number of semitransparent hairs, variable in size and in the form of their terminal cells, will be seen radiating from the edge of the section. Thin sections will show the mode of attachment of the base or "foot" of the hair to the epidermis of the stem. Selecting an uninjured and straight hair, examine its component cells under *high magnification.* Frequently the lower or basal cells of the hair will prove most suitable for this study. By carefully regulating the light and constantly using the fine adjustment on the microscope, the *nucleus* with its *nucleolus* will be visible. In color, the nucleus will appear gray and slightly opaque. Often the nucleus may appear to be imbedded in the thin layer of *cytoplasm* lining the wall of the cell. Commonly, however, the nucleus is suspended in various positions by a delicate and complex network of *cytoplasmic strands* that extend from the peripheral cytoplasm through the clear watery *cell-sap* of the prominent vacuome of the cell. In many of the cells of the hair, small *plastids* may be seen in the peripheral cytoplasm and sometimes in the larger cytoplasmic strands. Well-mounted uninjured sections that have not been allowed to dry out are suitable for a study of the *streaming movement of the cytoplasm* as well as changes in the relative position of the nucleus in the hair cells (cf. Seifriz, 1943 for a review of the phenomenon of protoplasmic streaming).

2. *Plastids.* Specialized cytoplasmic bodies known as plastids are commonly found in many types of plant cells. In meristematic cells and tissues that are not exposed to direct illumination, the plastids are usually devoid of pigment and are termed *leucoplasts.* A special type of starch-forming leucoplast, which occurs commonly in food-storage tissue, is called an *amyloplast.* Under appropriate stimuli, e.g., light, a part or all of the leucoplasts of a cell may develop chlorophyll pigments and become *chloroplasts.* Another extremely common type of plastid is the *chromoplast,* frequently responsible for the yellow, orange, and yellow-red color of certain petals, fruits, and roots. Similar colors, however, may be due to *anthocyanin pigments* in the cell-sap, and hence the cytological basis for color in each unknown case can only be determined by direct investigation. Although the role of chloroplasts in photosynthesis is well known, the function of

many chromoplasts is obscure. In the case of the biologically important *carotene* in the cells of carrot roots Weier (1942, p. 35) states: "The pigment is present in discrete crystal-like bodies, associated with starch grains in a complex, resembling plastids, and in irregular small patches of cytoplasm." Excellent summaries on the development and structure of plastids are given by Weier (1938) and Sharp (1943, pp. 29-34).

Obtain a leaf from the outer region of the terminal bud of *Anacharis* and mount it carefully in a drop of water. Examine this leaf first under low magnification, noting that the thin lateral flaps at either side of the "midrib" are composed of only two layers of essentially similar cells. In short, there is no mesophyll as distinct from an epidermis. Study a number of cells in this leaf at varying depths of focus under *high magnification.* Note that all cells contain numerous, small, discoid *chloroplasts* which typically are in a peripheral position. Many cells, at least in healthy leaves, will exhibit cytoplasmic streaming. Careful examination will show that, although the chloroplasts are nonmotile in themselves, they are passively carried in a clockwise or counterclockwise direction by the circulating cytoplasm. Often the chloroplasts are so numerous in a cell that they mask the nucleus. The latter can be more easily observed in the more transparent teeth-like cells that occur irregularly at the margins of the leaf. *Prepare* water mounts of tomato fruit, carrot root, asparagus and rose "hips" and examine them under low magnification. Note the wide variation in size and shape of the chromoplasts.

3. *Ergastic substances.* All living cells in plants contain variable amounts of non-protoplasmic materials that may be collectively designated as *ergastic substances.* They include storage products, waste material, or by-products of protoplasmic activity and in elementary botanical texts are often termed "inclusions." One of the most common examples of ergastic substances is the *vacuome,* which consists of a dilute aqueous solution of a wide range of inorganic as well as organic materials.

In addition to the vacuome, many types of plant cells contain further ergastic material in the form of *reserve food* such as starch, proteins, ar[illegible]ts or oils. Perhaps the most common type of insoluble food is *starch,* which occurs as grains, the size

and form of which are highly specific. *Obtain* a thin, transverse section of the stem of *Pellionia* and, after mounting it in water, examine the large parenchyma cells of the cortex under *low and high magnification.* Most of these cells contain starch grains that have developed within the chloroplasts. Often, "fragments" of the chloroplast may be seen at the broad end of the pear-shaped starch grains. The addition of dilute iodine to the section will give the blue color reaction typical for starch. (Cf. Weier, 1936, for a detailed study of the structure of the chloroplast in *Pellionia pulchra.*) In the tissue of such storage organs as tubers, fleshy roots and cotyledons, starch grains are formed by the activity of *amyloplasts.* These starch-forming plastids lack chlorophyll and are to be regarded as a specialized type of *leucoplast. Secure* a small amount of fresh potato tissue and after gently teasing it with dissecting needles, mount it in water, add dilute iodine and examine under *low magnification.* Note the numerous obovoid starch grains in every cell. Examine a single starch grain under *high magnification* and observe, near the smaller end of the grain, the minute refractive point termed the *hilum.* Careful regulation of the light and patient use of the fine adjustment will usually reveal a number of more or less distinct *eccentric layers* arranged about the hilum. For comparative purposes, examine the storage parenchyma cells in the bean cotyledon, noting the difference in the form and position of the hilum in the ovoid starch grains.

A very common type of ergastic substance in many kinds of plant cells is *calcium oxalate,* which appears usually in the form of conspicuous well-defined *crystals.* (For extensive treatments of crystals, cf. Netolitzky, 1929 and Pobeguin, 1943.) It is generally held that such crystals represent an excretory product of the protoplast, being formed by the union of calcium with oxalic acid. The recent monograph by Netolitzky (1929), however, reveals that substances other than oxalic acid may combine with calcium to produce crystalline bodies. Comparatively little is known concerning the factors, chemical and biological, that regulate the rate and mode of crystallization, and that hence determine the form of the adult crystal. Netolitzky (1929, p. 47) concludes that in the final analysis it is "the nucleus which de-

termines which form of crystal will be produced, perhaps by regulating the velocity of crystallization within the cell itself." Examples of the three main forms of plant crystals may now be studied.

(a) *Druses* consist of more or less spherical aggregates of sharp-pointed angular crystals, the whole mass often suggesting in its form the mace-head of medieval warfare. Comparatively few intensive studies have been made on the origin and development of this type of crystal. According to Scott (1941, pp. 226-230) the druse in *Ricinus* is evident first as a single crystal of variable shape suspended in the cytoplasmic strand of a young, vacuolating cell. The mature druse originates either by the aggregation of separate crystals or by dendritic growth. As the druse reaches its adult form and size, it becomes enclosed within "a cellulose sheath and anchored to the cell wall by one or more hollow cellulose stalks" (Scott 1941, Fig. 11, 20, 21). To study druses, *examine* fresh sections of stems and petioles of *Ricinus* or *Rumex*. Prepared transections of the stem of *Pelargonium* likewise are useful. A comparison of longi- and transverse sections will show that the crystal-containing cells often occur in files or vertical rows, each cell of which may develop a single druse (cf. Scott 1941, Figs. 18-19).

(b) *Raphides* are long, slender, needle-shaped crystals that may occur as solitary structures in a cell but more commonly develop in definite bundle- or sheaf-like groups. Although such crystals appear most abundantly in the tissues of monocotyledons, in certain dicotyledonous families such as the Oenotheraceae (Netolitzky 1929, p. 48) raphides are virtually a diagnostic character. *Obtain* a living plant of duckweek (*Lemna* sp.) and mount it in water under a cover-glass. Note under *high magnification* that many of the surface cells at the margins of the thallus-like "leaves" contain prominent bundles of raphides. The root tip of *Lemna*, particularly the root cap and the cortex, is particularly favorable for a study of raphides, especially if viewed under *polarized light*. For comparison, *examine* under low magnification freshly cut longisections of the stems of *Tradescantia* or *Zebrina*, noting the much larger raphides, many of which may be displaced from the cells during the cutting of the sections.

Thick sections, however, may be used to demonstrate raphides *in situ.*

(c) *Prismatic crystals,* which often occur singly in cells, are most commonly found in parenchyma cells of the xylem and phloem. Scott (1941, p. 230) found that in *Ricinus* "the mature solitary crystal is inclosed within a heavy cellulose sheath, practically fused to the cell wall or visibly attached by one or more very short stalks." *Study* prepared longisections of the phloem in the stem of *Pinus,* noting the large prismatic crystals in many of the phloem parenchyma cells.

III. Suggested Drawings and Notes

1. Prepare an enlarged drawing of a single hair of squash, petunia, or *Tradescantia* (staminal hair) as seen under low magnification, showing accurately the number and form of the cells of which it is composed and its mode of attachment.

2. Draw a single living cell of a hair as it appears under high magnification. This drawing should portray a "median optical view" and should include the following: cell wall, nucleus (and its visible parts), cytoplasm, vacuome, plastids. *Record,* as laboratory notes, all observations made on cytoplasmic streaming and nuclear "movement" in the material studied.

3. Select a cell from the "midrib" region of the *Anacharis* leaf and prepare drawings to show its appearance and contents as seen in surface and median optical views. Describe concisely the variations in the rate and direction of cytoplasmic streaming in cells in different regions of the leaf. What may be the physiological significance of cytoplasmic streaming? (Cf. Seifriz, 1943, pp. 93-98.) Summarize the evidence indicating that plastids do not arise *de novo* in the cytoplasm of plant cells (cf. Weier 1938, pp. 518-522, and Sharp 1943, pp. 33-34).

4. Prepare drawings to illustrate the form and the arrangement of chromoplasts in the material studied.

5. Draw cells from the cortex of *Pellionia,* the potato tuber, and the bean cotyledon showing the size and form of the included starch grains.

6. Prepare drawings to show the form and position of the various types of crystals studied.

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Exercise II

THE CELL WALL

Sperms, eggs, and certain other cells of the gametophyte of angiosperms are naked protoplasts and show no evidence of a definite cell wall. Save for these particular exceptions, the zygote and all the succeeding generations of sporophytic cells derived from it are provided from the beginning with a cell wall. Indeed, the rigid and often massively thickened cell wall is frequently cited as a significant distinction between plants and animals; the cells of the latter are either naked protoplasts or else possess thin and less clearly demarcated walls, features obviously related to the marked pliancy of many animal tissues.

One of the most apparent concomitants of the differentiation of meristematic into "mature" tissue consists in a series of complex changes in the structure and usually the chemical nature of the cell walls. As the individual cells enlarge, the original walls necessarily increase in area and, in many cell types, increase more or less conspicuously in thickness. Pronounced thickening of the wall is followed in certain types of cells, e.g., tracheids, most fibers, cork cells and vessel elements, by the collapse and ultimate disintegration of the protoplast. In the final phases of development of vessels and articulated laticiferous tubes a considerable portion of the end walls of the component elements is dissolved away, leaving *perforations*. Descriptive histology has exploited in great detail the diversified structural and chemical patterns assumed by the cell wall in an effort to classify morphologically cell types and tissues. Furthermore, an understanding of the physical and chemical nature of cell walls is intimately related to the proper interpretation of such physiological processes as growth, absorption, and translocation. It is evident, therefore, that knowledge of the fundamental nature of the cell wall is indispensable in the investigation of the development, structure, and physiological activities of plant tissues.

Within the last two decades, the classical concepts of wall

structure and development have been re-examined with the aid of new or improved techniques. In addition to the use of reagents that swell, selectively stain, or remove certain parts of the cell wall, the application of X rays, microincineration, and polarized light represents new lines of attack on the problem.

In the present exercise, the salient features of cell-wall structure and development will be summarized briefly and illustrative laboratory material will be presented. For detailed reviews of the present status of knowledge on cell walls the student should consult the papers of Kerr and Bailey (1934), Bailey and Kerr (1935), and Bailey (1938, 1940).

I. The Gross Layers of the Cell Wall

The walls separating the protoplasts in embryos and apical meristems typically are thin and, unless examined with the aid of special techniques (e.g., under polarized light), may appear homogeneous and unstratified. Recent investigations, however, show clearly that the cell walls in all meristems, including the vascular cambium, are tripartite, consisting of a median *intercellular layer* bounded on each side by the *primary walls* of adjacent cells. In the differentiation of many cell types, such as photosynthetic parenchyma, epidermal cells, and collenchyma, the original primary wall increases more or less conspicuously in area and thickness but no additional major wall layers are formed. Hence the original tripartite condition is "carried over" from the meristematic to the functionally specialized cells and may be demonstrated readily with appropriate techniques. The maturation of many cell types, however, is accompanied by the deposition of a supplementary wall termed the *secondary wall.* Examples are provided by tracheary elements, fibers, and sclereids. In cells of this type, the protoplasts in the cell lumina of adjacent cells are separated by a *complex of layers* consisting of the middle lamella, the two primary walls, and the two secondary walls. Much confusion has existed in the past with reference to the interpretation and terminology of the gross or major layers of cell walls, and such terms as "middle lamella," "primary wall," "secondary wall," and "tertiary wall" have been used inconsistently and ambiguously by various writers. Kerr and Bailey

(1934), as a result of an extensive study of material with modern techniques as well as on the basis of a critical survey of the literature, have clarified the entire problem. Their general interpretation and proposed terminology, which have been widely adopted (Anderson, 1935; Hayward, 1938; Foster, 1942; Sharp, 1943; Eames and MacDaniels, 1947), will be followed throughout this book.

1. The *intercellular layer* or *middle lamella.* As Kerr and Bailey (1934) have emphasized, the term "middle lamella" should be applied only to the layer of truly *isotropic pectic material* that is situated between the primary walls of tissue elements. Although the early phases of development of this intercellular layer are poorly understood, it appears to originate from the "transformation" of the cell plate during the final stages of cytokinesis (cf. Eames and MacDaniels, 1947, pp. 24-27). Structurally regarded, the intercellular layer is amorphous and, according to Kerr and Bailey (1934, p. 344), "possesses few of the attributes of a true membrane." The plasticity of this layer appears to facilitate the varied types of adjustment which arise during cell division and cell enlargement. In many instances, as in the thick-walled cells of wood, the intercellular layer becomes highly lignified.

2. *The primary wall.* This term should be restricted to the original individual wall of a meristematic cell and its homologues in differentiated tissues. In contrast to the isotropic middle lamella, the primary wall is *anisotropic,* when viewed under polarized light, and consists of a continuous system of microfibrils of cellulose. Within the elongated interstices of this matrix there may occur the most varied substances, among the most common of which are pectic compounds, lignin, cutin, and suberin. The primary wall is rarely uniform in thickness throughout its extent but, on the contrary, exhibits thin areas arranged in a great variety of patterns. These areas are known as *"primary pit fields,"* and within them groups of delicate *plasmodesmata* are usually found. Primary walls, even in functionally specialized tissue, retain to a remarkable degree their capacity for reversible changes in thickness and for renewed growth in area. This plastic nature of the primary wall has been particularly emphasized by Bailey (1940) and is one of

the factors that permits many types of "mature" cells to resume growth or division when properly stimulated. Unless special technique is adopted (e.g., the use of polarized light) the intercellular layer and its thin adjoining primary walls may appear as a single non-lamellated partition. It has been suggested that for convenience in description, the term "*compound middle lamella*" may be applied to this tripartite complex. The usefulness of this term will depend however, as pointed out by Kerr and Bailey (1934, p. 342), upon the accuracy with which a given technique is able to discriminate between the true primary walls and the immediately adjacent portions of the secondary walls.

3. *The secondary wall.* This layer, which is typically the most massive of all the gross layers, is produced usually after a cell has reached its final size and form. Bailey (1938, 1940) maintains that, unlike the primary wall, the secondary wall is "apparently incapable of growth or of increase in surface area." Hence, in his view, cells that develop true secondary walls are unable to resume growth when stimulated "unless the protoplast can escape from its indurated envelope." Many fibers, which retain their protoplasts, become septate as a result of the subdivision of the cell cavity but this is not accompanied by any appreciable change in the size of the "mother cell." On the other hand, Bailey's viewpoint is in opposition to the observations of certain other investigators who contend that cells with thick lignified walls are capable of growth and "dedifferentiation." Bloch (1941, p. 120), for example, who has reviewed this problem, states: "In lignified vessels, sclerenchyma, and endodermal cells of various monocotyledons, wounding seems rather frequently to induce dedifferentiation." Evidently further intensive study of the developmental potencies of the varied types of living thick-walled cells is urgently needed to clarify the problem.

The secondary wall, structurally and chemically, is highly complex. Like the primary wall it consists of a coherent matrix of cellulose within which a wide range of compounds may occur. A discussion of the extensive range of *visible structural patterns* in the secondary wall is beyond the scope of this book (cf. Bailey and Kerr, 1935). Suffice it to state that this wall is very often

conspicuously stratified and that the distinctness of the successive lamellae depends upon either physical or chemical deficiences or upon a combination of both. Whether the thick secondary wall is formed by the process of *intussusception* (the intercalation of new "micellae" of wall substance among those present) or by *apposition* (the centripetal deposition of successive layers or lamellae) is not entirely clear. It is possible that both processes are involved to varying degrees. Bailey (1940) maintains "that the classical controversy regarding growth by intussusception versus growth by apposition will ultimately be settled largely in favor of apposition." The secondary wall is rarely continuous over the entire surface of the adjacent primary wall. In tracheary elements of the primary xylem, for example, the secondary wall is developed as discrete rings, spiral bands, bars, or as a complex reticulum, while, in many cell types, well-defined *pits* occur. In certain tracheary elements, the pitted secondary wall is overlaid by a distinct series of helical bands. The latter were formerly designated as representing a *"tertiary wall"* but morphologically they appear to be part of the secondary wall (cf. Eames and MacDaniels, 1947, p. 30).

II. Intercellular Spaces

The enlargement and morphological specialization of tissues in plants is very commonly accompanied by the progressive development of *intercellular spaces* between the cells. In tissues where the spaces at maturity appear as small triangular- or diamond-shaped cavities (e.g., thin-walled parenchyma, collenchyma) it seems to have been assumed in the past that they originated simply by the splitting of the middle lamella at the corners between enlarging cells. The intensive researches of Martens (1937, 1938), however, show that the process is more complex, especially in the case of dividing cells with relatively thick primary walls. According to Martens, when such a cell divides, the new middle lamella at first comes into contact not with the old, external, middle lamella but with the inner surface of the primary wall. Thus the new and old middle lamellae are at first separated by a cellulosic wall. At the level of intersection of the new middle lamella, a small *intramembrane cavity* appears and,

developing centrifugally, eventually reaches the outer middle lamella, thus creating (in sectional view) a triangular cavity (cf. Eames and MacDaniels, 1947, p. 29, Fig. 16). Very often, the origin of the intramembrane cavity is preceded, accompanied, or followed by the development of a separate cavity within the old middle lamella—this cavity is truly intercellular. The mature space may thus result from the confluence of two distinct cavities, one formed within the cellulosic primary wall, the other within the old middle lamella. With reference to extremely thin-walled cells, such as occur in the immediate vicinity of apical meristems, the demonstration of intramembrane cavities is difficult and certainly requires further research (cf. Priestley and Scott, 1939). But Martens' studies have placed the entire problem in a new light and should stimulate comparative investigations. Among the many unsolved problems is the question of the way in which the destruction of localized regions of the middle lamella and primary wall occurs. Whether this is brought about by a "gelification" of the pectic compounds, accompanied by physical stretching and tearing, is still uncertain. For a detailed treatment of the origin, distribution, and function of air spaces in plants the student should consult the review by Sifton (1945).

III. Plasmodesmata

The protoplasts of adjacent cells of a wide variety of plant tissues have been shown to be interconnected by delicate strands of cytoplasm termed *plasmodesmata*. As stated previously, plasmodesmata are characteristic features of primary cell walls within which they fluctuate widely in abundance and distribution but are very commonly aggregated in clusters in the primary pit fields. In cells that develop secondary walls, plasmodesmata appear to be restricted to the closing membranes of pit-pairs. Protoplasmic connections seem to support the idea that the cell wall, at least during certain stages of its growth, is an integral part of the living system of the cell and not merely a "lifeless" excretion of the protoplast. Knowledge as to the origin and development of plasmodesmata is still meager (Meeuse, 1941) but there is some indirect evidence that additional "secondary" protoplasmic connections may arise during the development of

elongating fibers and ramifying sclereids. In such cases, portions of the cell establish new cell contacts by "gliding" or "intrusive" growth between neighboring elements, and during this phase new plasmodesmata may originate (for further information cf. Meeuse, 1941 and Foster, 1944, p. 320). Although Meeuse (1941, p. 259) concludes that "the true functions of plasmodesmata have not been definitely determined," there is considerable evidence as to the physiological significance of these structures. Haberlandt (1914, pp. 635-638) suggests (1) that they may be essential in transmitting external and internal stimuli through plant tissues and (2) that in storage tissue, such as endosperm, they may be "principally concerned with trans-location." Furthermore, it has been suggested that plasmodesmata are the channels through which certain economically important viruses may travel. Lastly, it is probable that the high degree of correlation between the cells, tissues, and organs of the plant may depend upon the presence of plasmodesmata. Livingston (1935), who maintains that plasmodesmata occur in all the living tissues of the tobacco plant, concludes that "the evidence presented by numerous investigators indicates that actual protoplasmic connections between cells, or plasmodesmata, are generally present throughout all living tissues of higher plants, thus establishing the organism as a definitely correlated entity of interconnecting protoplasts, instead of a community of separate cells."

IV. Material for the Study of Wall Layers and Plasmodesmata

1. *Wall layers.* Meristems and thin-walled parenchyma tissue should be used to study cells devoid of secondary walls. *Examine* a stained section of a root or shoot apex, noting the thin, apparently homogeneous "walls" separating the protoplasts. Under *polarized light*, these tripartite walls will appear more or less birefringent (i.e., bright) because of the anisotropic nature of the *primary walls*. The use of fresh, unstained sections is usually needed, however, to bring out the distinction between primary walls and the middle lamella. The *parenchyma tissue* of the cortex and pith of stems (e.g., *Pelargonium* and *Tilia*) furnishes illustrations of mature cells that lack true secondary walls and

that are separated from one another by *intercellular spaces.* The origin and early development of intercellular spaces may be readily studied from serial trans- and longitudinal sections of root tips.

The stem of the widely cultivated Wax Plant (*Hoya carnosa*) provides excellent material for a study of the origin and structure of the *secondary wall. Secure* a transection of the stem (several internodes distal to the terminal bud) and note the varied stages of development of sclereids in the pith. The sclereids originate from the "secondary sclerosis" of certain of the pith cells as the result of the deposition of a thick, clearly laminated, secondary wall. During secondary-wall development in the sclereid, conspicuous *canal-like pits* are formed. If *Hoya* is not available, the structure and, if possible, the development of the secondary wall in fibers (e.g., the phloem fibers of *Linum, Nicotiana* or *Tilia*) should be studied.

2. *Plasmodesmata.* Endosperm tissue of certain seeds provides useful material for a study of the general features of plasmodesmata. *Obtain* a prepared slide of the endosperm tissue of the persimmon (*Diospyros*) and examine the section under *high magnification.* Note that the greatly thickened primary walls are traversed by solitary or spindle-shaped groups of very delicate "lines" which are the plasmodesmata. According to Quisumbing (1925), the plasmodesmata are numerous in the walls of the endosperm of *D. discolor* and *D. Ahernii* whereas in *D. kaki* and *D. ebenaster* "they are few, restricted and grouped at the walls. They occur single or in groups of two, three, four, five or six, and are thicker when single and usually thinner in groups."

V. Pits

With few exceptions, the secondary wall of plant cells is interrupted by small cavities or recesses termed *pits.* These structures vary widely in size, arrangement, and form, but since they exhibit some constancy in a given cell type they provide significant criteria in comparative studies, especially of tracheary elements. Pits typically occur in pairs; i.e., a cavity in the secondary wall of a cell normally lies opposite a similar recess in the adjacent cell. Hence the term *"pit-pair"* appropriately

designates the usual condition and is contrasted in meaning with *"blind-pit,"* which is a pit "without complement opposite to an intercellular space" ("Glossary of Terms Used in Describing Woods," p. 5). In the simplest case, each member of a pit-pair consists of (1) the *pit cavity*, which is the actual recess left within the secondary wall, and (2) the *pit aperture* or opening from the cell lumen into the pit cavity. Separating the members of a pit-pair is a distinct *pit membrane* devoid of secondary wall layers and consisting of the middle lamella and the primary walls of the two connected cells. From a *functional standpoint*, pits are believed to facilitate the movement of substances between cells.

In the strict sense, pits or pit-pairs are structures restricted to cells which develop true secondary walls and which, therefore, should not be confused with the thin *primary pit fields* typical of the cells of meristem and certain of their derivatives. This morphological concept, advocated in the "Glossary of Terms Used in Describing Woods" (p. 4) and followed in a number of texts dealing with wood anatomy (Record, 1934, Brown and Panshin, 1940), is based fundamentally on the *method of development of pits*. These structures are laid down during cell differentiation within the primary pit-fields, as a result of the interrupted deposition of the secondary wall. As the latter is built up, one or more pit cavities develop within the confines of each primary pit-field. Typically, these cavities are paired between adjacent cells, but in the case of *"unilaterally compound pitting,"* one pit may have two or more smaller complementary pits in the adjacent cell. The investigations of Bailey and Faull (1934) illustrate the theoretical importance of distinguishing between pits and primary pit-fields. They found that the cell walls of the vertical wood parenchyma and wood rays in *Sequoia sempervirens* are entirely primary in nature and are provided with more or less conspicuous primary pit-fields. The latter only superficially resemble the true pits occurring in the secondary walls of the xylem parenchyma and ray cells of members of the Abietoideae. Admittedly it is in practice often impossible (in the absence of ontogenetic study) to draw a rigid distinction between "pits" and "primary pit-fields." This difficulty possibly is responsible for the state-

ment in Eames and MacDaniels[1] (1947, p. 37) that "pits are structural features either of the primary wall alone or of the primary plus the secondary wall." Esau (1948, p. 234, footnote) has also proposed a less rigid use of the term "pit" in her recent treatise on the phloem of *Vitis*. In the present volume, the term "pit" will be used as far as possible in the strict sense defined above.

Pit-pairs may be conveniently classified under three major types viz.:

1. *Simple pit-pairs*, which are typical of cells that retain a protoplast throughout their functional life, are particularly well developed in parenchyma cells of the secondary xylem. In *face view*, the *aperture* appears as a circular, elliptical, or even irregular area. In macerated tissue,[2] simple pit-pairs in this view appear as refractive red points of light. A recognition of this optical characteristic will help to distinguish simple pits from particles of protoplasm or other substances lying free in the cell lumen. In *sectional view*, the *cavity* of each member of the pit-pair is often of equal diameter throughout and there is no overarching rim or border produced by the adjoining secondary wall. Examples of the range in variation of the form of the cavities of simple pit-pairs are given by Eames and MacDaniels (1947, p. 39, Fig. 23).

2. *Bordered pit-pairs* are typical of water-conducting cells, such as tracheids and vessel elements, but also occur in modified form in the thick walls of wood fibers. In such conifers as *Pinus*, a highly developed type of bordered pit-pair develops between the tracheids. The large circular cavity of each member of the pair is overarched by a rim-like extension of the secondary wall termed the *pit border*. The apertures of such bordered pits are circular or oval in contour. As seen in median sectional view, the border overarching each pit cavity is apparent, and an additional structural peculiarity is observable, namely, the *torus*. The latter is a discoid, thickened area of the pit membrane which exceeds the diameter of the pit aperture. The thinner peripheral

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[1] By permission from *An Introduction to Plant Anatomy*, 2nd ed., copyright 1947, McGraw-Hill Book Co., Inc.

[2] Cf. Appendix, pp. 214-216 for the technique of macerating plant tissue.

portion of the pit membrane of *Pinus* and certain other conifers is elastic and under such conditions the torus may be tightly pressed against one of the apertures of the pit-pair. The bordered pits of tracheary elements in other vascular plants vary in form, structure, size, and abundance within wide limits and provide diagnostic characters in the morphological study of woods. For details the student should consult Record (1934), Brown and Panshin (1940), and Eames and MacDaniels (1947).

In many types of tracheids, fiber tracheids and wood fibers, the secondary walls are exceedingly thick and the structure of the bordered pit-pairs correspondingly modified. In these cases, two apertures are present in each pit, viz.: (1) the *outer aperture*, which leads directly into the small, circular, deeply imbedded *pit chamber*, and (2) the *inner aperture*, which opens into the cell lumen. The inner aperture moreover is elliptical or, in extreme instances, slit-like in form and may extend considerably beyond the limits of the pit cavity when the wall is studied in face view. Such apertures are termed *"extended"* and are a characteristic feature of many thick-walled tracheary elements and fibers. The inner and outer apertures are connected by a *pit canal*, which has the form of a compressed funnel. (For a three-dimensional reconstruction of this modified type of bordered pit, cf. Eames and MacDaniels, 1947, p. 47, Fig. 30.) Sometimes the extended inner apertures of the two members of this type of bordered pit-pair coincide in orientation. Frequently, however, the two apertures are crossed.

"Vestured pits," which were discovered and named by Bailey (1933), are distinguished from all other bordered pits by the possession of minute outgrowths on the sides of the pit chambers and at the edges of the pit apertures. (For illustrations, cf. Eames and MacDaniels, 1947, p. 47, Fig. 31.) Vestured pits have been observed in only a relatively few families of the angiosperms and represent important diagnostic characters in the comparative study of woods.

3. *Half-bordered pit-pairs* represent "an intercellular pairing of a simple and a bordered pit" ("Glossary of Terms Used in Describing Woods," p. 5) and occur when parenchymatous cells develop in contact with tracheary elements. According to a frequent opinion expressed in texts, the pit member on the side

of the living cell is simple whereas its mate on the side of a tracheid or vessel is bordered. Frost (1929), however, in a study of the nature of pitting between tracheary and parenchymatous cells in angiosperm xylem, has found that this conception has no general validity. He concludes that "fully bordered, half-bordered and simple pits are characteristic features between tracheary cells and vascular parenchyma" and that "the type of pitting on the wall of the parenchyma cell is controlled largely by the degree of specialization of the vessel or fiber which lies next to it." Furthermore, in the case of the wood-ray-tracheid connections in *Sequoia*, the term "half-bordered pit-pairs" is not strictly applicable since, according to Bailey and Faull (1934, p. 242), the investigator "is dealing with bordered pits which have no complementary pits on the ray side." Obviously the whole question of pitting in plant cells demands further investigation, from both a comparative as well as an ontogenetic point of view.

VI. Material for the Study of Pits

Simple pit-pairs are well developed in the living parenchyma cells of the secondary xylem of many dicotyledons and gymnosperms. *Obtain* a preparation of macerated wood (*Tecoma radicans, Vitis, Quercus* or *Abies* provide excellent material) and examine it under *low magnification,* noting the numerous parenchyma cells. These cells are box-like in form and occur singly or in series, depending upon the extent to which the xylem has been macerated. Study a connected group of parenchyma cells under *high magnification* and investigate the size, structure, and position of the simple pit-pairs as seen in surface and sectional views.

Bordered pit-pairs are highly developed in the tracheary elements of the secondary xylem of conifers. *Obtain* a preparation of macerated xylem of the stem of *Pinus* or *Abies* and examine it *under low magnification.* Bordered pit-pairs are large and obvious in the tracheids, which are elongated cells with acute or blunt tips. Select a suitable tracheid and study the appearance of the bordered pits in *face view under high magnification.* Observe that each pit appears as three concentric outlines. The

outermost circle demarcates the edge of the pit cavity, the intermediate circle represents the edge of the torus, and the somewhat refractive innermost circle is the pit aperture. In many tracheids, the successive bordered pits are more or less clearly set apart from each other by "eye-brow" or rim-like ridges, termed *crassulae*. These are interpreted as "thicker portions of the intercellular layer and primary walls between primary pit-fields" and in the past have been designated as "Bars of Sanio" and "Rims of Sanio" (cf. "Glossary of Terms Used in Describing Woods," p. 4). The structure of bordered pit-pairs in sectional view can be effectively studied in macerated material if the *edge* of the pitted walls are turned towards the observer. In order to study critically the pit membrane and the torus, it is necessary to examine thin, properly stained, tangential and transverse sections of pine xylem.

The specialized type of bordered pit with slit-like extended inner apertures is clearly illustrated in the fiber tracheids and fibers of the wood of many dicotyledons. *Obtain* a preparation of macerated secondary xylem of sycamore (*Platanus*) and examine it under *low magnification*, noting the numerous long, acuminate *wood fibers*. Careful study of these fibers under *high magnification* will reveal, in face view, more or less numerous bordered pits with narrow extended apertures and inconspicuous circular chambers. Occasional fibers may be turned in such a way that the pits, with their characteristic *canals*, may be seen in sectional view. A study should also be made, using the oil-immersion lens, of the structure of this type of bordered pit-pairs in prepared, stained longisections of the wood of *Platanus* or some other suitable dicotyledon. When the crossed slit-like inner apertures of bordered pit-pairs are seen in face view, they often present characteristic "X-shaped" figures.

The secondary xylem of *Pinus* provides material for a study of ***half-bordered pit-pairs***. These structures develop at the points of contact between tracheids and ray parenchyma. *Radial sections* reveal the large "window-like" apertures characteristic of *both* members of such pit-pairs. *Transverse sections* will show (a) the wide *pit membrane*, devoid of a torus, which often bulges into the lumen of the tracheid, and (b) the simple, unbordered

character of the pit in the contiguous ray cell. On the tracheid side, a very narrow, rim-like border is present.

VII. Suggested Drawings and Notes

1. Prepare drawings to illustrate the structure of a small group of cells from the apical meristem of a root or shoot.

2. Draw a group of pith or cortical parenchyma cells from the transection of the stem of *Tilia* or *Pelargonium*, showing and labeling the following: *compound middle lamella, protoplast, intercellular spaces.*

3. Draw several stages in the development of the secondary wall of the sclereids in the pith of the stem of *Hoya carnosa.* Show and label the following: *primary wall, primary pit fields, intercellular spaces, secondary wall, pits.* If *Hoya* is not available, draw a group of phloem fibers of *Tilia* as seen in a transection of the stem showing and labeling the following: *compound middle lamella, secondary wall, lumen.*

4. Prepare a drawing to illustrate the arrangement of the *plasmodesmata* in a small group of endosperm cells of *Diospyros. Write* a brief summary of the various explanations that have been made regarding the origin and development of plasmodesmata (cf. Meeuse, 1941, pp. 255-258).

5. Draw several connected wood parenchyma or wood-ray cells from the macerated xylem of *Tecoma* or *Abies*, showing the size, structure, and arrangement of the *simple pit-pairs* as seen in face and sectional views. Label the following: *compound middle lamella, secondary wall, pit aperture, pit cavity, pit membrane.*

6. Draw, on a large scale, a single tracheid from macerated pine xylem, showing the form and arrangement of the *bordered pits.* Prepare drawings based on a study of longitudinal and transverse sections of pine wood, illustrating the structure of *bordered pit-pairs.* In these drawings, label each of the following (when it is present): *compound middle lamella, secondary wall, pit border, pit aperture, pit cavity, pit membrane, torus, crassulae.*

7. Prepare a drawing to illustrate the half-bordered pit-pairs that occur at the points of contact between the wood ray and the

adjacent tracheids in the stem of *Pinus*. Represent the pitting as seen both in the transverse and radial planes of section.

8. Prepare drawings, based on a study of macerations and longisections of the wood of *Platanus*, showing the structure of the modified *bordered pit-pairs* developed in the fibers. Label the following: *compound middle lamella, secondary wall, outer aperture, inner aperture, pit chamber, pit canal.*

9. Prepare a summary of the investigations of Anderson and Kerr (1938) on the development and structure of the primary and secondary walls of cotton hairs.

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Exercise III

MERISTEMS

In marked contrast to animal development, all vascular plants exhibit an "open system" of growth consisting of the formation of new organs and tissues throughout the life of the individual. This distinctive kind of growth depends fundamentally upon the maintenance of regions of dividing cells at certain specifically restricted portions of the shoot and root. Such regions of theoretically unlimited growth and cell division are termed *meristems*. The term "meristem" was originally introduced by Nägeli (1858) to designate a type of "dividing tissue" composed of parenchymatous cells, which Nägeli regarded as morphologically distinct from the "cambium." In modern usage, the vascular and cork cambia, as well as the so-called "growing points" of shoots and roots, are included under the collective term of *meristem*. The significance of meristems in the "open system" of growth of the plant is most vividly shown by long-lived woody perennials in which each season's growth is accomplished by the formation of a crop of new shoots, reproductive structures, and roots as well as by an increase in diameter of the distal portions of stems and roots. From the standpoints of organography and comparative morphology, however, it is evident that the *relative duration* of cell formation in meristems fluctuates within wide limits. The apical meristems of the vegetative shoots and roots in many gymnosperms and arborescent dicotyledons may appear capable of indefinite life and self-perpetuation. Actually, of course, various factors, such as malnutrition, insufficient water, injury by cold, insects or pathogens, may result in the death of an apical meristem. Furthermore, the phenomenon of *correlation*, in this case involving the relative persistence of the apical bud as compared with the lateral buds, may profoundly influence the behavior of terminal meristems in the shoot system. Comparably, the functional life of the vascular cambium is subject to many internal and external influences. In certain trees, this lateral

meristem may continue to form phloem and xylem tissues for hundreds or even, as in *Sequoia*, for thousands of years. But in many herbaceous plants and in certain parts of woody plants, the functional life of the cambium may be limited to a single season of growth. Obviously the maintenance of an indeterminate type of meristem, such as a "growing point" or a cambium, reflects the existence of a physiological state in such regions quite unlike that present in mature tissues. In the growth of determinate organs, such as leaves, the leaf-apex is maintained for only a brief period in the state of a meristem and the cells soon enlarge and cease division. Evidently the factors, genetical and physiological, that permit the maintenance of meristems at strictly localized regions in the plant are complex and deserving of critical study. The study of the phenomenon of "dedifferentiation" (cf. Buvat, 1944, 1945), further experiments with "tissue cultures" (cf. White, 1943, 1946 and Gautheret, 1945), and the recent cultural and experimental study of the apical meristem of shoots (cf. Ball, 1946, 1947 and Wardlaw, 1947) may be expected to contribute significantly to a better knowledge of the factors that condition the kind of cell activity typical of meristems. For an interesting treatment of the "causal" aspects of meristems, the student should consult the articles by Linsbauer (1916) and Priestley (1928).

I. Meristems and the Process of Cellular Differentiation

The origin of new cells from a meristem and their gradual enlargement and morphological-physiological specialization represent interrelated processes which may be conveniently designated by the word "differentiation." From a *purely descriptive standpoint*, the differentiation of any particular tissue has been characterized as a "progressive" loss of the embryonic features of meristem-cells and the progressive attainment of the state of "maturity." Two unfortunate results of this viewpoint are: (1) the assumption that a valid *morphological distinction* can be drawn between typical meristem-cells and the varied categories of living cell types and (2) the use of the term "permanent" to designate cells distal to a meristem (cf. Eames and MacDaniels, 1947, p. 61). With respect to the first assumption, it is now clear

that undue emphasis has been placed in the past on such cytological features as the degree of vacuolation, the presence of inclusions, and the relative thickness of the wall in an effort to segregate meristem-cells from their derivative tissue elements. The cytological investigations of Bailey (1930) on the cambium and recent studies on the structure of cells in apical meristems (Zirkle, 1932) have demonstrated that *all* meristem-cells are vacuolated to varying degrees and may contain inclusions of various sorts. Furthermore, the *primary walls* of many types of meristems may be excessively thick and provided with conspicuous primary pit-fields. In such cases, the meristem-cells cannot be separated, on morphological characters, from such cell types as parenchyma or collenchyma. With regard to the term "permanent," it is now clear that this word can only be applied to such irreversibly specialized cell types as enucleate sieve-tube elements, and to such dead tissue elements as tracheids, vessels, and cork cells. All other living nucleated cells, whatever may be their form, wall structure, and position, retain a marked capacity for growth, cell division and "redifferentiation" under proper stimuli (Bloch, 1941; Buvat, 1944, 1945). Thus, in plants, tissue differentiation is not simply the progression from a juvenile state to the condition of irreversible specialization. On the contrary, most tissues in plants contain a relatively high proportion of cells that have retained all the capacities for division and growth possessed by "typical" meristem-cells. The "potencies" of a living cell, therefore, cannot be determined by morphological analysis but only through experimental investigations. Work along this line has revealed a remarkable range of performance on the part of many cell types and suggests that the terms "differentiation" and "dedifferentiation" are in urgent need of clarification (cf. Buvat, 1944, 1945). If a living epidermal cell, under proper stimulus, can resume division and contribute to the formation of a bud (Hartsema, 1926; Naylor and Johnson, 1937), it seems questionable whether such an element should properly be designated as "differentiated," at least from a physiological standpoint. The same question can be properly raised with respect to the infinitely varied types of parenchyma cells which likewise possess a remarkable capacity for further growth and specialization. (Cf.

Bloch, 1941; Buvat, 1944, 1945; Gautheret, 1945; White, 1943, 1946 for further information and literature.)

II. Apical Meristems

One of the most notable aspects of modern plant anatomy is the widespread revival of interest in the fundamental problems of cellular structure and growth posed by the apical meristems of shoot, root, and flower. It must be emphasized, however, that these problems are not the product of recent investigations, but that, on the contrary, they occupied the attention of some of the most notable botanists of the past century (for reviews of the classical studies on the apical meristems of shoots, cf. Schüepp, 1926; Foster, 1939, 1941, 1943; and Wardlaw, 1945). Much of the work of the 19th century was centered upon the determination of the number of "*initial cells*" in apices and the origin of tissue from them. The "apical cell theory" of Nägeli (1878) and Hanstein's (1868) "histogen theory" represent two of the major conflicting interpretations that emerged from this type of inquiry. During the past decade, a much more comprehensive picture of apical meristems has developed than was possible with the limited knowledge and cruder techniques of the early workers. Many of the most recent investigations have been concerned with the varied "*zonal patterns*" of cell structure and activity in the shoot apices of gymnosperms and angiosperms. Other investigations have initiated the exploration of *floral histogenesis* with particular reference to the structure of the floral apex and the origin of floral appendages. The use of colchicine in the induction of *periclinal and sectorial chimeras* represents an entirely new approach to the classical problem of *cell specificity* in apical meristems, and the results obtained thus far are of considerable interest. Lastly, *experimental investigations* on the behavior of "isolated" shoot apices of lower vascular plants as well as angiosperms have emphasized the limitations of a purely morphological approach to the problems of organ and tissue determination (cf. Ball, 1946, 1947, and Wardlaw, 1947). A detailed treatment of these diversified investigations on apical meristems cannot be undertaken in this book. Indeed within the last ten years, more than fifty papers have appeared devoted largely to the subject of

the cytohistology of the shoot apex. In the brief résumé given below, no pretense, therefore, to a complete bibliography is made. But an effort has been made to include the most recent review articles which may serve as a guide to the voluminous literature and which will supplement the necessarily limited treatment given in the present volume.

1. *The shoot apex*

(a) *Introduction.* The classical investigations of C. F. Wolff (1759) on bud development showed that new leaves and new stem tissues are traceable in origin to the delicate tip of the shoot. Wolff designated this region as the "punctum vegetationis," a term which has been rather freely translated as the "growing point." Despite the widespread adoption by anatomists of the expression "growing point," this term carries an inaccurate implication and in the present book will be replaced by the more appropriate and noncommittal designation of "shoot apex." This decision is based upon the fact that the *chief significance* of the so-called growing point is that it represents the *region of initiation* of the primary organization of the shoot, rather than a localized area or "point" of "growth." As a matter of fact, if "growth" in a morphological sense is regarded as an increase in size of cells, tissues, and organs, this process is obviously at a minimum in the "growing point."

Great variation exists in the *form* and *dimensions* of the shoot apex of seed plants. As seen in median longisection view, the apex commonly has the form of a mound or low dome. In *Elodea, Myriophyllum,* and *Hippuris* and in many grasses, however, the shape of the shoot apex is that of a slender, blunt-tipped cone (cf. Louis, 1935; Sharman, 1945, 1947). The apex of dicotyledons with decussate phyllotaxis (e.g., *Syringa, Lonicera, Ligustrum,* etc.) is particularly suitable for developmental studies because the initiation of each pair of foliar structures is preceded by a notable and symmetrical *expansion* of the terminal meristem. Since this process is repeated each time a pair of leaves is produced, the apex exhibits a rhythmical alternation of what Schmidt (1924) called "minimal" and "maximal" areas (cf. also Louis, 1935, Pl. II, Figs. 20-21; and Cross, 1937, Figs. 10-11). This situation emphasizes the fact that the form and the dimensions of

the shoot apex are likely to vary, depending upon whether an active or dormant apex is measured as well as upon the particular phase in shoot development under examination. Extremely few careful measurements have been made of the shoot apex of seed plants, and no generalizations are possible at present. Although the angiosperms typically possess rather small apices that range in diameter from 90 μ in certain grasses to 130-200 μ in the dicotyledons, the apices of certain palms and of *Trichocereus* are respectively 500 μ and 700-900 μ in diameter (Ball, 1941b; Boke, 1941). In the gymnosperms the shoot apex appears to fluctuate significantly in size and form as is shown by the recent tabular summary compiled by Kemp (1943, p. 509). The apices of conifers tend to be relatively narrow and conical in form with dimensions resembling those in the angiosperms. But in *Ginkgo* and especially in the cycads, the apex is much broader and the diameter may be 3-8 times that of the height (cf. Johnson, 1944b). In *Cycas revoluta,* the shoot apex during bud expansion attains the relatively enormous diameter of 3.5 mm., a dimension greatly exceeding that recorded for any other vascular plant (Foster, 1940). The nature of the relationship between size and form of the apex, on the one hand, and the morphology and primary anatomy of the shoot, on the other, are obviously complex and await further comparative studies for their solution (Bower, 1930, Ch. XII; Foster, 1939, 1940, 1941).

(b) *Zonal structure of the shoot apex in gymnosperms.* According to the classical concept, the tissue composing the shoot apex of vascular plants represents a *"primordial meristem"* characterized by the "undifferentiated" homogeneous morphology of its cells. Modern explorations on the cytohistology of the shoot apices of a wide range of seed plants do not support this idea. On the contrary, with the aid of improved microtechnique, the so-called primordial meristem (or "promeristem" of many American authors) proves to exhibit a remarkably complex *zonation.* By the term "zonation" is meant the existence in the apex of definable regions or zones distinguished from each other by such features as (1) cell and nuclear size, (2) reaction to plasma and nuclear stains, (3) relative frequency of mitoses, (4) planes of cell division, and (5) relative thickness of cell walls.

Beginning with the discovery of the marked zonal pattern in the apex of *Ginkgo biloba* (Foster, 1938), an extensive series of investigations have been made on the apices of other gymnosperms. (Cf. detailed reviews by Foster, 1939, 1941; Kemp, 1943; Johnson 1944b, and Majumdar, 1945.) In *Ginkgo*, the cycads, *Sequoia* (Sterling, 1945), and *Pseudotsuga* (Allen, 1947b; Sterling, 1946), all the tissues of the shoot apex may be traced in ultimate origin to a terminal series of periclinally and anticlinally dividing surface cells; these collectively form the *initiation zone*. Perhaps the most striking feature of the apices of these plants, however, is the presence of a more or less conspicious *zone of central mother cells* distinguished by the large-size, prominently vacuolated cytoplasm and often thick primary walls of the component cells. From the sides and the base of the central mother-cell zone, by means of oblique and transverse planes of cell division, the remaining zones of the apex are produced. In *Ginkgo* and the cycads, these consist of the lateral *"peripheral zone"* and the basal *"zone of rib meristem."* The term "rib meristem," which was devised by Schüepp (1926, pp. 18-19), designates a meristematic tissue composed of vertical series of transversely dividing cells. A typical rib meristem is a conspicious feature of the terminal region of many shoots and is very clearly displayed in the lower portion of the shoot apex of *Ginkgo*, the cycads, and other seed plants (cf. Sinnott and Bloch, 1941). According to Sterling's investigations of *Sequoia* and *Pseudotsuga* (1945, 1946), a *zone of "eumeristem,"* composed of small, actively dividing cells, is interposed between the central mother cells, and the succeeding lower zones of pith mother cells and rib meristem. Allen (1947b) however interprets the basal zone of cells derived from the central mother cells as a typical rib meristem in *Pseudotsuga*. Our present comparative knowledge of the zonal structure of the shoot apex in other gymnosperms is still so incomplete that interpretation is difficult and the phylogenetic trends of specialization obscure. In *Torreya californica*, for example, the shoot apex, although exhibiting a well-marked zonation, lacks a clearly defined central mother-cell zone (Kemp, 1943). Various genera in the Taxodiaceae, according to the investigations of Cross (1939, 1941, 1942, 1943a, 1943b), agree in possessing a basic zonal pattern in

their shoot apices. The "typical" apex in this group of the conifers is described by Cross (1943a, p. 140) as consisting "of a small group of apical initials, a protoderm, a variable number of subapical mother cells, a peripheral meristem and pith mother cells." In marked contrast to the situation in *Ginkgo*, the cycads and a number of conifers, there appears to be a distinct *trend* in the Taxodiaceae toward the development on the flanks of the apex of a *discrete surface layer* which does not contribute cells to the inner zones. This is regarded by Cross (1943b, p. 346) as "the most unique single characteristic" of the shoot apex in this family. Whether the weakly stratified apex of members of the Taxodiaceae typifies a stage in the evolution of the *prevailingly* stratified apex of angiosperms is, of course, hypothetical and emphasizes the need for further explorations of zonal structure in the shoot apices of gymnosperms. However, it is interesting and possibly significant that, in the highly evolved Gnetales, the apices of *Ephedra altissima* (Gifford, 1943) and *Gnetum gnemon* (Johnson, 1947) are each provided with a well-defined external cell layer. In conclusion it should be emphasized that the distinctness and, to some extent, the type of zonation in the shoot apex vary with the phase of activity of the meristem. Thus in *Torreya*, *Sequoia*, and *Pseudotsuga*, the most conspicuous display of zones occurs during the period of foliage-leaf production (cf. Kemp, 1943; Sterling 1945, 1946; Allen, 1947b). Ideally, the interpretation of the zonal pattern in *any* apex should be based upon the ontogenetic development of the meristem from the embryo to the adult plant. Allen's (1946, 1947a, 1947b) work on *Pseudotsuga* and that of Miller and Wetmore (1945a, 1945b, 1946) on *Phlox* are good examples of this needed type of investigation.

(c) *Zonal structure of the shoot apex in angiosperms.* At the beginning of the modern period of cytohistological investigations of apical meristems, two very stimulating interpretations had already been proposed regarding the shoot apex of angiosperms, viz.: (1) the "histogen theory" of Hanstein (1868) and (2) the "tunica-corpus theory" of Schmidt (1924). Since both of these theories have been discussed critically in detail in a series of modern reviews (Foster, 1939, 1941; Sifton, 1944; Majumdar, 1945), it will only be necessary to outline very briefly their most

salient features. According to Hanstein, the shoot apex in angiosperms consists of a central core or "*plerome*" of more or less irregularly arranged cells enveloped by a variable number of mantle-like layers. The latter are subdivided into the outermost layer of the apex, termed the "*dermatogen*," and the underlying layer or layers, designated as the "*periblem*." In Hanstein's interpretation, dermatogen, periblem and plerome arise from separate sets of *initial cells*, and function as discrete "histogens" as follows: The dermatogen propagates the epidermis, the periblem gives rise to the inner tissues of the leaf, and the cortex of the stem and the plerome forms the vascular system and the pith. Although Hanstein's theory strongly influenced the study and interpretation of the shoot apex in both gymnosperms and angiosperms for many years, it ultimately proved inadequate for the following reasons, viz.: (1) in many angiosperm apices (and very obviously in the apices of gymnosperms as a whole) a clear distinction between a "periblem" and "plerome" does not exist, and (2) the respective roles in tissue-development assigned by Hanstein to the three histogens cannot be demonstrated (for further details cf. Foster, 1939, pp. 456-459). Schmidt's (1924) tunica-corpus theory, in contrast, is *noncommittal* as to the ultimate destiny of cell-lineages in the apex and recognizes two major zones, viz.: (1) the *tunica*, which collectively designates the layer or layers in the apex, in which the *anticlinal plane of cell division* predominates except at the points of origin of leaf and axillary bud primordia; and (2) the *corpus*, in which the planes of cell division fluctuate and result in a core of more or less irregularly arranged cells. Tunica and corpus reflect, respectively, *surface and volume growth* within the apex and it is evident from a number of studies that (1) the number of tunica layers varies widely between species and genera and (2) the sharpness of the boundary between tunica and corpus may fluctuate even within the same species at different phases of its ontogeny [1] (cf. Boke, 1940, 1947; Reeve, 1942, 1948; Engard, 1944). The tunica-corpus theory

[1] In certain grasses (Sharman, 1940, 1943) and in *Chlorogalum pomeridianum* (Sterling, 1944), periclinal divisions occasionally appear in the outermost cells of the shoot apex. This "gymnosperm-like" behavior lends further support to the belief that a rigid morphological treatment of tunica and corpus zones should be avoided.

therefore is adapted to a *flexible description* of cell arrangement in the apex (rather than to rigid morphological exploitation) and with but few exceptions (e.g., Sharman, 1945 and Dermen, 1947) is widely employed in modern literature (cf. Philipson, 1947; Reeve, 1948). When modern techniques are used, it is evident that the *cytohistology of the corpus* of the shoot apex of a number of angiosperms resembles to a marked degree the zonation typical of many gymnosperms. In the cactaceous genera *Trichocereus* and *Opuntia*, for example, Boke (1941, 1944) found that the corpus exhibits three zones distinguished by the size, structure, and planes of division of the cells. An apparently comparable zonation likewise occurs in the apices of certain palms (Ball, 1941b) in the bamboo *Sinocalamus* (Hsü, 1944) and in a series of dicotyledons which have been examined very recently by Philipson (1947) and Reeve (1948).

(d) *Apices of inflorescences and flowers.* It is only within the past ten years that the greatly neglected problem of *floral histogenesis* has begun to receive attention in the light of knowledge of the structure and growth of vegetative shoot apices. The most comprehensive study of the cytohistology of the floral apex and of the method of initiation of floral organs, especially the carpel, was made by Grégoire (1938). His conclusions, although rejected by many investigators, have nevertheless been of great service in stimulating intensive research on the similarities and differences in zonal pattern and growth between vegetative and flowering apices. (For reviews, cf. Foster, 1939; Brooks, 1940; Reeve, 1943; Engard, 1944; Joshi, 1947; and Philipson, 1947). Grégoire maintained that there is no counterpart, in floral apices, of the tunica-corpus zonation characteristic of the vegetative apex. On the contrary, the floral apex consists of a massive central core ("massif parenchymateux") of large, thin-walled, highly vacuolated cells enveloped by an extensive *mantle* ("manchon meristematique") of smaller and very actively dividing cells. From this mantle, in which the predominant plane of cell division is periclinal, the floral organs, their vascular traces, and the cells of the "massif parenchymateux" arise. This distinctive zonation is the basis for Grégoire's radical assertion that floral and vegetative apices represent two "irreducible types" of morphological

organization in the angiosperms. Two main objections have been raised against the views of Grégoire, viz.: (1) the zonal structure of the floral apices of a number of plants differs in no essential respect from that of their vegetative apices (Satina and Blakeslee, 1941). Fluctuations in the number of tunica layers may occur, but such variation may well reflect simply changes in the interrelationship between surface and volume growth rather than significant morphological differences (cf. McCoy, 1940; Sass, 1944; Miller and Wetmore, 1946; and Boke, 1947). (2) Grégoire failed to take account of the progressive changes in zonal structure that occur during the gradual differentiation of a terminal inflorescence or flower (Reeve, 1943; Philipson, 1947). Very recently Philipson (1947, p. 191) has suggested that Grégoire's "mantle" should be interpreted as equivalent to the *combined* tunica-corpus zones of a vegetative apex whereas the parenchymatous "core" represents merely the maturing pith cells of the inflorescence axis. A discussion of the bearing of histogenetic studies on the morphological interpretation of the flower lies beyond the scope of this book. For a full discussion of the problem the student is referred to the literature cited above, especially to the extensive discussion given by Engard (1944).

(e) *The origin and roles of the "primary meristematic tissues."* In this book the term "primary meristematic tissues" will be used collectively to designate those aggregations of cells, derived from the various zones of the apex, which give rise to the "primary body" of the shoot and root. From a purely topographical standpoint there is still much merit in the concept and terminology of Haberlandt (1914, pp. 94-98). He recognized three so-called "primary meristems," viz.: (1) the *protoderm*, from which the epidermis and variable amounts of internal tissue are derived. In angiosperms, the protoderm is the direct descendent of the outermost tunica layer of the apex, while in many gymnosperms it only appears as a "stabilized" layer at points relatively distal to the apex; (2) the *procambium*, composed of prosenchymatous cells from which, in Haberlandt's interpretation, the bulk of the vascular and fibrous "strands" of the adult plant arise. In more modern usage, the term "procambium" is restricted to that primary meristematic tissue from which only the primary vascular

system develops (cf. Esau, 1943a); and (3) the *ground meristem*, which is defined by Haberlandt as "the whole of the primary meristematic tissue that remains undifferentiated, after the protoderm and all the primary procambial strands have been segregated." From the ground meristem there originate varied tissues including the fundamental parenchyma of cortex, pith and leaf mesophyll as well as additional procambial bundles. The origin and role of the protoderm are usually readily investigated, but the mode of development of procambium and ground meristem from the internal zones of the apex, and the contribution of these primary meristematic tissues to shoot and root development are problems of great complexity which are in urgent need of study and clarification (cf. the detailed treatment of this subject by Esau, 1943a).

Very recently, the use of *colchicine* in the induction of polyploid cells in the shoot apex of several angiosperms has provided a new and very hopeful line of attack in the study of the origin and "fate" of cell lineages. The most comprehensive investigations have been carried on with *Datura* and include a study of the vegetative apex (Satina, Blakeslee and Avery, 1940), the origin of leaf and flower (Satina and Blakeslee, 1941), the development of the carpel (Satina and Blakeslee, 1943), the development of style, stigma, calyx and corolla (Satina, 1944), and the development of the ovule (Satina, 1945). Stated in briefest terms, the application of a dilute solution of colchicine to the shoot apex of *Datura* by inducing polyploidy resulted in a variety of *periclinal chimeras*, i.e., of apices in which the two tunica layers and the underlying corpus varied as to chromosome number and cell size. In some cases, for example, the outermost tunica layer consisted of tetraploid or octoploid cells while the second tunica layer and the corpus were composed of normal diploid cells. In other cases, the second tunica layer was polyploid while the surface layer and the corpus consisted of diploid cells (cf. Satina, Blakeslee and Avery, 1940, p. 902, table 1, for additional types). Because of the differences in size and chromosome number in the varied periclinal chimeras, the descendents of the diploid vs. the polyploid zones of both vegetative and floral apices could be traced. In short, it was possible to determine, with some precision, the relative con-

tribution made by the various zones of the apex to the differentiation of the primary meristematic tissues in leaves, floral organs, and stems. More recently, a similar type of investigation has been carried on by Dermen (1947) with reference to cranberry (*Vaccinium macrocarpon*). The apex of this plant, like that of *Datura*, exhibits a corpus enveloped by a two-layered tunica, and several different types of periclinal chimeras resulted from colchicine treatment. According to Dermen's study, the role of the various layers of the apex in histogenesis is much less precise than that reported for *Datura*, particularly with reference to the origin of the procambium and mesophyll in the leaf. The study of the apex and its derivative tissues in the varied types of chimeras of a variety of angiosperms would doubtless modify our present over-formalized conception of histogenetic processes (for a general review on chimeras cf. Jones, 1937).

2. *The root apex.* The apex of the root differs fundamentally from that of the shoot in the presence of a *root cap*. The latter is a thimble-shaped or conical structure that occupies the true physical apex of the root. It acts as a "buffer" for the delicate meristematic tissue, which is thus *subterminal* in position. Great variation exists in the histogenetic relationships between the root cap and the subterminal meristem. Indeed, the variations have resulted in the designation of a number of "types" of root apices, which are distinguished (1) by the mode of origin of the cap, and (2) the relation of the various so-called "histogens" to the origin of the primary meristematic tissues in the root proper. (Haberlandt, 1914, pp. 89-94, and Hayward, 1938, pp. 44-48.) It is an interesting fact that, although the highly deterministic scheme of Hanstein (1868) has been largely abandoned for the shoot apex, the structure and growth of the root apex are still generally interprcted in terms of Hanstein's "histogen theory" (Hayward, 1938; von Guttenberg, 1940, 1941; Eames and MacDaniels, 1947). While it is true that the absence of foliar structures and buds in roots makes it relatively easier to determine the point of origin of a given tissue, it may well be questioned whether Hanstein's concepts are any more justified for the root than for the shoot. This point of view receives support from the experimental studies of Brumfield (1943) on the root apices of *Crepis capillaris* and

Vicia Faba. X-ray treatment of the seedling roots of these plants resulted in chromosomal chimeras of the *sectorial type*, i.e., one or more *vertical* strips or sectors of tissue, including root cap, epidermis, cortex, and central cylinder, contained cells with aberrant chromosome morphology. Brumfield concluded that the whole root develops from a few (possibly only three) cells and that "sectorial chimeras would not be expected if the root meristem consisted of 'histogens' as described by Hanstein."

In the light of modern researches, it is clear that the present confusion as to the various so-called "types" of root apices results from (1) the failure to trace the complete history of the root apex from the embryo to the adult plant, (2) the failure to recognize possible differences in the details of cellular structure between the apex of the primary root and the apices of the lateral roots, and (3) the lack of broad knowledge concerning the details of cytohistological zonation in root apices. Allen (1946, 1947a, 1947b) has contributed to a more realistic knowledge of the root apex by his thorough study of the embryo and seedling of *Pseudotsuga* and similar investigations should be made on the root apices of angiosperms. Until more reliable data are available, it seems best to adopt the procedure of Esau (1940, 1943b), who designates the tissues derived from the root apex as root cap, epidermis (or protoderm), immature cortex, and vascular cylinder (or stele). In many dicotyledons the root cap and protoderm seem to originate from a common *initial zone*, whereas the cortex and central cylinder arise from apparently independent sets of initiating cells (e.g., *Raphanus* and *Linum*). In certain monocotyledons (e.g., *Zea* and *Triticum*) the root cap appears to develop from a distinct histogenetic layer or *"calyptrogen."* Other "types" of root apices are recognized (cf. Haberlandt, 1914; Hayward, 1938), but, for the reasons stated above, a careful reinvestigation of their zonal structure seems highly desirable.

III. The Vascular Cambium

The term "vascular cambium" is applied to vertical strips or narrow cylinders of enlarging and dividing cells that are *lateral* in position and that give rise to the secondary phloem and secondary xylem. In many herbaceous angiosperms, especially

many of the monocotyledons, and in most of the lower vascular plants, cambial activity is reduced or absent and the vascular system is therefore largely "primary" in character. In woody angiosperms and in the gymnosperms, however, the primary tissues of stem and root are short-lived and become destroyed or buried by the more massive secondary vascular system formed by the cambium.

The most significant of modern studies on the structure and growth of the cambium have been made by Bailey (1920, 1923, 1930), who has studied both fixed as well as living cells in a wide range of gymnosperms and angiosperms. From a morphological standpoint, the cambium may be regarded as a *single layer* of cells in which *tangential* (i.e., periclinal) *divisions* predominate during the propagation of phloem and xylem. Two principal types of *initials* occur in the cambium, viz.: (1) the *fusiform initial,* which as seen in *tangential* longisectional view is prosenchymatous in form and in certain plants, according to Bailey, may attain the enormous length of 5000 μ, and (2) the *vascular-ray initial,* which is a much smaller cell and is more or less isodiametric in form. The fusiform initials form such elements as tracheids, vessels, fibers, wood parenchyma, and sieve-tubes, whereas the ray initials are points of origin and propagation of the radially disposed phloem and xylem rays (cf. Barghoorn, 1940). One of the many interesting features of cambial cells is their highly vacuolate character, which is only evident when living tissue is critically studied with the aid of such vital stains as "Neutral Red." Bailey (1930, p. 677) states: "Normal cambial initials are conspicuously vacuolated. Indeed certain of them are as highly vacuolated as plant hairs, which are commonly cited as illustrations of extreme specialization of the protoplast in fully differentiated cells. The classical conception of non-vacuolated meristems, and the various physiological generalizations that have been deduced therefrom, should be abandoned." Just how the form, wall structure, vacuome, and peculiar methods of cytokinesis in cambial initials are related to the derivation in *opposite directions* of such *heterogeneous tissue systems* as phloem and xylem is not yet clear. It would seem evident, however, that here, as with comparable problems at the root and shoot apex,

experimental studies (e.g., tissue cultures and transplantation) may ultimately illuminate much of the obscurity of this important problem

IV. The Phellogen

From this lateral meristem are derived cork, phelloderm, and lenticels. A discussion and study of the phellogen will be given in the exercises devoted to the stem and the root (cf. Exercises XIII and XV).

V. Material for the Study of Meristems

1. *The shoot apex.* A proper study of the zonal structure of the shoot apex and of the origin of leaves, buds, and the primary meristematic tissues requires thin, *serial* longitudinal and transverse sections. The successful fixation, imbedding in paraffin, sectioning, and staining of apices are procedures that demand extreme care and skill. For detailed treatments of the subject of microtechnique for the shoot apex reference should be made to Ball (1941a), Johansen (1940), Sass (1940) and to the "Methods" described in the modern literature cited in this exercise. Since the choice of bud material will be governed by many factors, no detailed descriptions of specific shoot apices will be attempted. Instead, general recommendations, as to the advantages and special features of a few rather generally available types of apices, will be made.

(a) *Gymnosperms.* The shoot apex of the widely cultivated *Ginkgo biloba* provides exceptionally good material for a study of a well-defined type of zonation. The apices of the "long shoots" are particularly instructive with respect to the origin and protracted activity of a typical "rib meristem" (cf. Foster, 1938, pp. 546-550 and Gunckel and Wetmore, 1946). For comparison, a study should also be made of the shoot apex of some type of conifer, e.g., *Abies* (Korody, 1937), *Pseudotsuga* (Allen, 1947b; Sterling, 1946), *Sequoia* (Sterling, 1945) or *Picea* (Korody, 1937).

(b) *Angiosperms.* The mound or dome-shaped form of apex with several *tunica layers* is well illustrated in such genera as *Carya* (Foster, 1935), *Morus* (Cross, 1936), *Rhododendron*

(Foster, 1937), *Acacia* (Boke, 1940), *Datura* (Satina, Blakeslee and Avery, 1940), *Sambucus and Helianthus.* Aside from minor variations, the origin of leaf-primordia and the development of pith, cortex, and procambial strands are similar in these dicotyledonous genera. The structure of the shoot apex in monocotyledons and the active participation of the outermost tunica layer in leaf formation can be readily demonstrated from serial longisections of the shoot tips of such grasses as *Triticum, Zea* (Sharman, 1942), or *Agropyron repens* (Sharman, 1945).

2. *Apices of developing flowers and inflorescences.* Regardless of the specific plant or plants chosen, an effort should be made to study the nature of the "transition" from the vegetative to the floral apex. This requires serial longisections of typical vegetative apices and a series of stages in flower-bud development. In the light of Grégoire's (1938) survey, various members of the Ranunculaceae, such as *Ranunculus, Helleborus* and *Aconitum* provide good material. The developing inflorescences of various composites, such as *Bellis* and *Succisa* (Philipson, 1947) or of the common snapdragon (*Antirrhinum*), will furnish additional material for individual study or laboratory demonstrations.

3. *The root apex.* For reasons discussed earlier in this exercise (cf. p. 38) it seems unwise at present to classify root apices into a series of morphological "types." It is therefore recommended that the laboratory material for this topic consist of a few carefully selected examples of monocotyledonous and dicotyledonous root apices which will show (a) several different methods of origin of the root cap and (b) the histology and method of development of the protoderm, ground meristem, and procambium. The root apices of *Raphanus, Pisum, Sagittaria* and such grasses as *Zea* or *Triticum* provide good laboratory material (cf. Hayward, 1938).

4. *The vascular cambium.* Prepared trans- and longisections of portions of small stems of various gymnosperms and angiosperms are very useful but have the disadvantage of giving a very inadequate idea of the cytology of cambial cells. The most instructive and realistic views of cambial initials are secured from sections of *living material* cut with the aid of a sliding microtome. The cambium of *Pinus* is a good gymnospermous

type with greatly elongated fusiform initials, whereas *Vitis* or *Robinia* illustrate representative dicotyledonous cambia.

VI. Suggested Drawings and Notes

1. Prepare *outline drawings* of the shoot tips of the various gymnosperms and angiosperms studied. In each outline, indicate diagrammatically by means of legends, the position and extent of *each* of the following *when* they occur in a given case: initial, central mother, peripheral and rib meristem zones; tunica, corpus, leaf primordium, axillary bud primordium, procambial strand, cortex, epidermis, pith.

2. Prepare outline drawings of the shoot apices of (a) *Ginkgo* or a conifer and (b) an angiosperm, filling in the cells of the various zones. Also show the form and arrangement of cells in a small portion of a procambial strand and several successive stages in the development of the rib meristem.

3. Prepare drawings to illustrate the form and cellular structure of the floral or inflorescence apices studied.

4. Draw in outline the entire longisection of one or more of the root apices studied, filling in the cellular details of representative portions of the root cap, initiation zones, protoderm, ground meristem, and procambium.

5. Prepare drawings of the vascular cambia studied, showing the structure in transverse, radial and tangential planes of section. In the drawings of the transverse and radial sections, a few layers of the most recently developed phloem and xylem tissues should be included.

6. Outline the processes of mitosis and cytokinesis as they occur in the dividing fusiform cambial initials of gymnosperms and angiosperms (cf. Bailey, 1920, 1923).

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Exercise IV

PROBLEMS IN THE CLASSIFICATION OF CELL TYPES, TISSUES, AND TISSUE SYSTEMS IN PLANTS

A correct morphological interpretation of the cellular structure of modern plants should rest ideally upon a full knowledge of the evolutionary development of cell types and tissues. Although such cell types as parenchyma, epidermal cells, guard cells and tracheids were well defined in the relatively unspecialized Psilophytales (Hoffmann 1934, pp. 237-240), the phylogeny of the highly complex tissues and tissue systems of living tracheophytes is obscure. As a consequence, our present concepts and terminologies, with reference to cell types and tissues, have arisen largely from the study of *modern highly evolved plants*. Early investigations were primarily concerned with the description and cataloguing of the diverse forms of cells. But during the latter half of the past century, the *ontogenetic* and *physiological* methods of approach supplanted this purely descriptive method and resulted in many of the current schemes for tissue classification. At the present time, the nomenclature and classification of cell types, tissues, and tissue systems are unfortunately in a confused and uncertain state. In the present exercise, a critical résumé of the most important schemes of classification is offered as a guide for the student in the interpretation and evaluation of modern histological literature. It is necessary to emphasize that the discrepancies in terminologies and in viewpoints to be discussed below have more than a mere historical interest. On the contrary, these differences underlie the varied presentations of plant anatomy in modern texts and continue to influence the modern trends in comparative and developmental histology. It is hoped, therefore, that the present critique may serve (1) to emphasize the provisional nature of *all* schemes of classification and (2) to direct attention to the need for a complete re-examination of the fundamental assumptions upon which these attempted classifications inevitably rest.

I. Classification of Cell Types

In the light of modern knowledge, many difficulties are encountered if one attempts to devise a comprehensive and satisfactory classification of cell types. From a dynamic standpoint, it is clear that a great many of the "mature" cell types of descriptive anatomy, as already pointed out (cf. Exercise III, p. 27), retain a remarkable capacity for growth, cell division, and further morphological specialization under appropriate stimulation (cf. Küster, 1908, p. 518 et. seq.). Cells of this sort (e.g., epidermal, parenchyma, collenchyma, endodermal, "pericyclic" cells) can therefore be segregated from meristem-cells only on the basis of size, form, and physiological activity and hence clearly are not irreversibly "differentiated" as to "type." Vöchting (1908, pp. 100-101), as one of the pioneers in the experimental study of plant histology, expressed the opinion that living plant cells do not possess "specificity" (a similar conclusion was reached by Küster, 1908, pp. 518-519). On the contrary, as shown by his experiments, all tissues of the plant body of kohlrabi "can develop directly or indirectly from pith-cells: *in a direct sense,* the elements of the primary cortex, thin-walled chlorophyll-containing parenchyma, sclerenchyma, collenchyma, bast, and idioblasts of the most varied form; *in an indirect sense,* by means of a cambium, the tissue forms of the xylem, vessels, tracheids, wood cells, sieve-tubes, companion cells; and finally a complete epidermis (can be formed) indirectly from the phellogen." Vöchting's general point of view is supported by the extensive range in behavior of various categories of living cells under the stimuli of insect or fungus attack (Küster, 1925), wounding (Bloch, 1941; Simon, 1908), and physiological isolation (Ball, 1946, 1947; Gautheret, 1945; Wardlaw, 1947; White, 1943, 1946; Buvat, 1945).

Because of the manifold potencies of many kinds of living cells, it is evident that all present schemes of classification are to a large extent artificial and suited primarily to the needs of descriptive histology (cf., for example, the artificial classification of cell types proposed by Lundegårdh, 1922, pp. 111-113). Indeed, it is only in the case of such irreversibly specialized elements as tracheids, vessel elements, enucleate sieve-tube elements, and

certain fibers that thorough morphological and even statistical treatment can be attempted (cf. Exercises X and XI). A more flexible and natural classification of both living and dead cell types doubtless will be possible when our present limited knowledge of the structure, physiological functions, and developmental potentialities of plant cells has increased. Meanwhile, however, the *practical needs* of the student can be met most effectively by means of a *tabular summary* of the morphological, topographic and dynamic features of the main kinds of cells recognized in modern descriptive histology. The tabular conspectus, appended at the end of the present exercise, makes no pretense to "completeness"; spores, gametes, and many types of physiologically specialized living cells have been deliberately omitted. But it is hoped that the conspectus may prove helpful for easy reference and that it may also serve as a guide to the terminologies of current anatomical literature.

II. Classification of Tissues

The varied morphological and physiological types of cells in vascular plants are usually organized into more or less well-defined cellular aggregations or "tissues." In the case of the primary body of the root and shoot, for example, the cell aggregates derived from the protoderm, ground meristem, and procambium soon become well defined both topographically and morphologically as "primary tissue systems." In a comparable manner, the cells derived from the vascular cambium form coherent masses of phloem and xylem tissue.

The historical development of plant histology illustrates that the most varied criteria have been adopted as the basis for defining and classifying tissues. De Bary (1884, p. 5), for example, maintained that the classification of tissues "must in the first place be founded on their structure, that is, on the structure of the single tissue elements, and the connection of these with one another." From this standpoint he distinguished the following six forms of vegetative tissue, viz.: (1) *Cellular tissue*, under which are included cell aggregates which retain their protoplasts, e.g., epidermis, parenchyma, collenchyma, endodermal layers, and cork; (2) *Sclerenchyma*, e.g., fibers and stone cells; (3) *Sec-*

retory structures; (4) *Vessels;* (5) *Sieve-tubes;* (6) *Milk-tubes.* In the light of our present knowledge it is clear that De Bary's scheme was highly artificial and that the presence or absence of a protoplast in a given mature cell type is not a reliable basis for morphological definition and segregation. A more recent example of the emphasis on morphological characters in classification is provided by the concept of "simple" and "complex" tissues proposed by Eames and MacDaniels (1947, p. 82). Simple tissues are homogeneous and consist of one kind of cell; complex tissues (e.g., xylem and phloem) are heterogeneous and contain several cell types. Such a distinction cannot be consistently carried out with reference to the tissues in higher plants, principally because few if any tissues *are* simple, either morphologically or physiologically. Parenchyma, it is true, is often more or less structurally homogeneous but very commonly *idioblasts* (i.e., *isolated* and *highly specialized cells*) are scattered within this tissue. Sclerenchyma, which is cited by Eames and MacDaniels as an example of a "simple tissue," may either occur as more or less uniform masses of fibers or sclereids, or either of these component cell types may develop as idioblasts in an otherwise homogeneous tissue (cf. Exercises VIII and IX).

In contrast to the purely morphological method of classifying plant tissues, a strong modern trend towards physiological and teleological interpretation was initiated by the work of Schwendener (1874) and Haberlandt (1914). The latter has assumed that the differentiation of specific cell-aggregates or "tissues" in plants is "mainly the outcome of division of labor, and that consequently the most characteristic features of each tissue are those which are most intimately connected with its physiological activity." Haberlandt's concept is thus essentially based on the interrelationship between the structural and functional aspects of tissues and is not primarily concerned either with ontogeny or phylogeny. A more complete discussion of his scheme of classification will be given in the following section of this exercise.

As Haberlandt (1914, pp. 69-70) has justly pointed out, the "aims of the particular investigator will largely determine the class of feature selected in a given case for purposes of definition and classification." But in the present author's opinion, there

has yet to be devised a satisfactory classification of tissues that will take into full account not only the structural and functional aspects of tissue-elements but also the marked capacity for many living cells in tissues to resume growth, cell division, and differentiation. Doubtless, as in the case of cell types, the fields of pathological and experimental anatomy will contribute significantly to the development of a more realistic grouping of plant tissues.

III. Classification of Tissue Systems

On the basis of topographical continuity or physiological similarity, the tissues of the plant body have been segregated by various anatomists into *tissue systems.* The classifications of tissue systems proposed by Sachs (1875) and by Haberlandt (1914) are outstanding because of their influence on the development of histological concepts and terminologies and for this reason will be considered in some detail.

1. *Sach's classification.* Sachs assumed that in the phylogenetic development of the higher plants from simpler multicellular forms, a distinction gradually arose between the outer layers of cells and the internal mass of tissue. The latter ultimately became differentiated into internal strands of cells surrounded by "fundamental" tissue. The final result of such evolutionary specialization is shown by the primary structure of the leaf, stem, and root, which consist, according to Sachs (1875, pp. 77-109), of *three principal systems of tissues,* viz.: (1) the *Epidermal System,* under which are grouped the epidermal and cork layers, (2) the *Fascicular System,* which contains the conducting xylem and phloem tissues, and (3) the *Fundamental Tissue System.* The latter was defined by Sachs "as those masses of tissue of a plant or of an organ which still remain after the formation and development of the epidermal tissue and fibro-vascular bundles." Sachs (*op. cit.*, p. 103) emphasized that his classification was not concerned basically with the forms (i.e., the morphological types) of cells "but with the contrast of different systems of tissues, each of which may contain the most varied cell forms." In view of the modern interest in the developmental potencies of living cells, it is interesting to note that Sachs avoided a rigid distinc-

tion between "permanent" and "meristematic" tissues. On the contrary, he emphasized the ability, especially of the cells of the epidermal and fundamental tissue systems, to reassume the state of a "formative" or dividing tissue.

Sach's classification of the adult vegetative tissues of higher plants into three main topographical systems is appealing in its apparent simplicity and practicability. That it does indeed possess considerable didactic value is shown by its adoption (with or without minor changes) in several modern textbooks (Sinnott, 1946; Molisch, 1927). From a more technical standpoint, Sach's classification is also used by Jeffrey (1917, pp. 8-13), who emphasizes, however, that the structural boundaries between the three tissue systems are more evident in the lower than in the higher vascular plants. Furthermore, the limits of the systems appear less sharply defined in the stem than in the "more conservative" root and leaf. In organs where secondary growth is prominent, the boundaries between the fascicular and fundamental systems may disappear and "in such cases the limits of the tissues can only be inferred from comparative and developmental anatomy."

The chief objection that has been repeatedly raised against Sach's classification is concerned with the indefinite physiological as well as morphological nature of the fundamental tissue system. As De Bary (1884, p. 6) pointed out, this system includes a wide range of tissue types and hence is not strictly co-ordinate with the "positively characterized" tissue forms of the dermal and fascicular systems. The concept of the fundamental tissue system has also been criticized on physiological grounds by Haberlandt (1914, p. 712). He states that this system includes "green photosynthetic parenchyma, colorless water-tissue, storage-parenchyma, mechanical strands and cell masses, endodermal layers, and the multifarious tissues which make up pericarps and seed coats." No one, therefore, will venture to maintain that "ground-tissue" constitutes "a whole of definite physiological character."

Despite the limitations of Sach's scheme, it does provide, especially *for the beginner in anatomy*, an extremely helpful frame of reference within which the more technical problems of cell and

tissue morphology can be studied. Furthermore, from an histogenetic point of view, the protoderm, procambium, and ground meristem represent, in a general sense, the precursors respectively of the dermal, fascicular and fundamental tissue systems. As Miller and Wetmore (1946, pp. 8-9) have pointed out, this fact should greatly facilitate the study and description of the ontogeny of the primary body in vascular plants.

2. *Haberlandt's classification.* Probably no scheme for classifying plant tissues has been carried out so consistently from a single point of view or in such detail as Haberlandt's "Anatomico-Physiological Classification." According to Haberlandt's viewpoint, the "principal function" should be the *sole guide* in the designation of any specific tissue "system." The "principal function" of a tissue is defined (*op. cit.*, p. 63) as "that form of physiological activity with which its most obvious and important anatomical features are correlated." The application of this idea resulted in the distinction by Haberlandt (1914, pp. 71-72) of twelve "anatomico-physiological tissue systems," each of which is typified by one major or "principal" function: e.g., absorption, conduction, protection, support, etc. With reference to the merits of his scheme of classification, he contends that "the anatomico-physiological definition and arrangement of tissues provide the broadest and most natural of all systems of tissue classification, since from this point of view the plant-body is regarded not merely as a more or less complex aggregate of formal elements, but also as a living organism, composed of a number of functional units and engaged in a corresponding number of physiological activities, which all contribute to the safety and welfare of the whole."

Haberlandt's high estimate of the value of his method for classifying tissues has been amply justified by its wide adoption in elementary as well as more advanced treatises on plant histology. Tschirch (1889) and Palladin (1914), for example, follow Haberlandt's system with little modification, and Molisch (1927) champions its merits for the advanced student. In this country, the anatomico-physiological classification has likewise proved popular and is utilized, in a somewhat simplified form, in such a recent compendium as Hayward (1938). But one of the

most significant illustrations of the prestige and influence of Haberlandt's ideas and classification is furnished by the ambitious "Handbuch der Pflanzenanatomie," which treats of the varied phases of anatomy in monographic form. Linsbauer, as the original editor, states in the first volume of this encyclopaedic work, that, aside from certain disagreement in details, the principles of Haberlandt's physiological anatomy will be adopted. In this same volume, an able and penetrating discussion of the various concepts and classifications of tissues is given by Lundegårdh (1922). This author, although agreeing in principle with the anatomico-physiological method of classification, emphasizes (*op. cit.*, p. 176) the need for a cautious and critical approach to the problem, since "the physiological-anatomical systems only indicate the normal combination of structure and function and obviously do not permit of any teleological conclusions as to the method of their origin." Lundegårdh (*op. cit.*, p. 175) proposes the following anatomico-physiological conspectus of tissue systems, viz.:

I. The Coherent Tissue Systems (composed of continuous cell aggregates)
   A. The Formative or Meristematic Tissues
   B. The Mature Tissues
      1. Systems with *dynamic functions*, e.g., assimilation, respiration, translocation, storage, absorption, etc.
      2. Systems with *static functions*, e.g., protection, mechanical support, etc.

II. The Disperse Systems (composed of *isolated* cells or cell-groups distributed as "islands" in the midst of various "coherent systems")
   A. Stomata (i.e., guard and accessory cells)
   B. Organs of Perception
   C. Reproductive Apparati
   D. Idioblasts (e.g., isolated sclereids)

Two principal objections have been advanced against Haberlandt's scheme of classification and the fundamental assumptions upon which any anatomico-physiological system is based. First of all, Haberlandt's system is constructed with respect to the

nature of the "principal function" of each tissue system. However, many tissues or cell types carry on more than a single function. In such instances, a distinction between a "principal" and a "subsidiary" function appears somewhat arbitrary. In other words, certain types of cells might with equal justification be classed in more than one of the anatomico-physiological "systems." Furthermore, as Lundegårdh admits, the principal function of a tissue (e.g., storage of solids) can be *deduced* only rarely from observation alone. Hence, any anatomico-physiological classification has a provisional character and is destined to be changed or modified in the light of new experimental data.

In the second place, the objection is raised that, in such a scheme as Haberlandt proposes, confusion results because of the disregard of the origin of cells and tissues. For example, in Haberlandt's classification, epidermal and cork cells, although differing fundamentally in origin, are grouped for physio-anatomical reasons under the "Dermal" or protective system. Conversely, guard cells and root hairs, while having a common origin from the embryonic surface cell-layer or "protoderm," are classified because of functional differences in the "Ventilating" and "Absorbing" systems respectively. In short, as Haberlandt (*op. cit.*, p. 70) emphasizes, to the physiological anatomist "the homologies of tissues are of no interest . . ." in defining and classifying the various tissues of the plant body; ". . . his concern is solely with analogy." Whether such a viewpoint leads to a "natural" insight into the evolutionary development of plant tissues is open to serious question. Jeffrey (1917, p. 8) says in this regard that "from the point of view of the doctrine of descent, functional features are of less significance, since it is precisely these which are the most readily modified and as a consequence furnish the least valuable indications of the course of evolutionary development in any given large group."

SUMMARY OF MAIN CELL TYPES IN SEED PLANTS

Cell Type	*Origin*	*Topography*	*Structural Characteristics*	*Functions*
Apical Meristem (Exercise III.)	More or less direct, lineal descendents of cells of the embryo, except in case of adventitious roots and shoots.	Apices of vegetative shoots, inflorescences, and developing flowers; in root, beneath inner edge of root cap.	Polyhedral; primary wall thin or irregularly thickened; nucleus large, ovoid; cytoplasm vacuolated to varying degrees; mitochondria, plastids and storage products may be present.	Point of origin of the primary meristematic tissues, e.g. (protoderm, ground meristem and procambium) from which the "primary body" of shoot and root develop; in shoot the tissue from which foliar primordia originate.
Vascular Cambium (Exercise III.)	From the procambium, from interfascicular areas in the vascular cylinder, and from reactivated parenchyma.	*Lateral*, in stem and root, between young phloem and xylem tissues.	Two types of initials, viz.: *fusiform*, and *ray*. In both, cytoplasm highly vacuolated; storage products may be present; primary pit fields conspicuous.	Produces secondary phloem and secondary xylem.
Epidermal (Exercise V.)	Protoderm	Surface cells (including trichomes) of foliar organs and of young stems and roots.	Epidermal cells variable in shape; guard cells of stomata often reniform; outer walls overlaid by cuticle and often cutinized; protoplast active, vacuolated, plastids present.	Mechanical protection; restriction of transpiration; aeration by means of stomata; photosynthesis; storage of water and products of metabolism; retain capacity for growth and division; common point of origin of adventitious buds.

Summary of Main Cell Types in Seed Plants

Cell Type	*Origin*	*Topography*	*Structural Characteristics*	*Functions*
Parenchyma (Exercise VI.)	Ground meristem, procambium, vascular cambium and, in the case of phelloderm, from the phellogen.	Widely distributed throughout plant body; may constitute the dominant tissue in cortex, pith and mesophyll; present in secondary phloem and secondary xylem as vertical strands and as vascular rays.	Vary in shape from approximately tetrakaidecahedral to stellately branched and cylindrical forms; primary wall with pit-fields, secondary wall present or absent; protoplast active and retains capacity for growth and division.	Photosynthesis; food and water storage; conduction; prominently concerned in wound healing and the origin of adventitious roots and buds.
Collenchyma (Exercise VII.)	Ground meristem of leaf and stem; in case of collenchymatous bundle caps, from procambium.	Hypodermal strands or cylinders in cortex of stems and petioles and ribs of foliage leaves.	± elongated, prismatic, often septate, with irregularly thickened primary walls, rich in pectin and with high % of H_2O; protoplast retains capacity for growth and division. Chloroplasts may be present.	Support of young stems and leaves.
Sclereid (Exercise VIII.)	In surface layer of seed coat from protoderm; idioblastic types from ground meristem or from parenchyma; may arise late in ontogeny from "secondary sclerosis" of parenchyma.	Diffused in cortex, phloem, pith and mesophyll as idioblasts or cell-clusters; in the laminae of some genera terminal on the vein endings; may constitute large part of seed coat and pericarp of fruits; prominent in bark.	Polyhedral, columnar, or ± profusely branched; secondary wall massive, usually of lignified cellulose and often provided with ramiform pits; protoplast may be retained at maturity.	Produces hard incompressible texture.

SUMMARY OF MAIN CELL TYPES IN SEED PLANTS

Cell Type	*Origin*	*Topography*	*Structural Characteristics*	*Functions*
Fiber (Exercise IX.)	Protoderm, ground meristem, procambium and vascular cambium.	Cortex, primary and secondary phloem, xylem; constitutes hypodermal strands or layers and the sclerenchymatous sheaths of vascular bundles in leaves; may occur as idioblast.	Typical example of prosenchymatous cell, often attaining considerable length; secondary wall usually thick, often highly lignified; pits sparse; lumen continuous, ± occluded or septate; protoplast usually absent at maturity.	Support.
Tracheid (Exercise X.)	Procambium and vascular cambium.	Primary and secondary xylem.	Prosenchymatous without distinct end walls; secondary wall of lignified cellulose, deposited as rings, spiral bands, transverse bars, or a reticulum, or is continuous except for pits; protoplast usually absent at maturity.	Conduction of water and certain solutes; support.
Vessel Element (Exercise X.)	Procambium and vascular cambium.	Primary and secondary xylem, occurring as ± extensive series of interconnected cells, each series termed a *vessel*.	Prosenchymatous to cylindrical, with distinct perforated end walls; perforations either simple, scalariform or reticulate; lateral secondary walls of lignified cellulose, with same range of pattern as in tracheid; protoplast absent at maturity.	Conduction of water and certain solutes.

Summary of Main Cell Types in Seed Plants

Cell Type	*Origin*	*Topography*	*Structural Characteristics*	*Functions*
Sieve-tube and Element Sieve Cell (Exercise XI.)	Procambium and vascular cambium.	Primary and secondary phloem; in angiosperms usually in lateral connection with companion cells and occurring in vertical series, each series termed a *sieve-tube.*	Prosenchymatous to cylindrical; end and often lateral walls provided with sieve areas; protoplast at maturity is enucleate.	Conduction of organic solutes.
Laticiferous Tubes (Exercise XII.)	Non-articulated type from initial cells which are devoid of septa; articulated type from dissolution of end walls of a continuous series of cells.	Cortex, phloem, xylem rays, pith, mesophyll; non-articulated type usually extensively branched; articulated type often interconnected by anastomoses, thus forming a net work.	Contain latex and are multinucleate; in non-articulated type, may continue apical growth and branching throughout life of the plant.	Storage of various food and waste products and possibly conduction.
Cork (Exercises XIII and XV.)	Phellogen	Peripheral regions of stems, roots and certain fruits; frequent in bud scales; often produced as a result of wounding.	Tabular, compactly arranged without air spaces; secondary wall suberized and usually devoid of pits; protoplast usually absent at maturity; lumen often contains crystals or tannin.	Restriction of transpiration. Cork tissue likewise is impervious to diffusion of gases except through lenticels.

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Exercise V

THE EPIDERMIS

From a purely topographical standpoint, the term "epidermis" may be applied to the superficial layer of cells in young stems and roots and in foliar structures. Since the epidermis represents, in this sense, the point of direct contact between the plant and its external environment, it is not surprising that this tissue exhibits considerable diversity in its structure and functions. Haberlandt (1914, p. 102) has proposed a restricted physiological definition of the epidermis that would include only "those superficial cells or cell-layers, the histological features of which clearly indicate that their principal function is that of a primary tegumentary or dermal system." According to this viewpoint, absorbing hairs and stomata would be excluded on physiological grounds from the epidermis. But as Linsbauer (1930, pp. 4-5) has clearly pointed out, it seems hardly justifiable to place the chief emphasis on the function of "protection" in the definition of the term epidermis. On the contrary, the cells that are morphologically a part of the epidermis may perform varied functions, important among which are mechanical protection, restriction of transpiration, water storage, aeration, storage of various metabolic products, absorption and photosynthesis. To subdivide such a "continuous" layer as the epidermis into various "anatomico-physiological systems" is more likely to result in confusion than is the broader topographical-morphological concept expressed above. The ontogenetic development of the epidermis likewise justifies its interpretation as a "morphological unit," since its origin is traceable to an external embryonic layer or *protoderm*. In lower vascular plants and in many gymnosperms, the protoderm of the shoot appears some distance from the summit of the apex as a superficial layer derived from the periclinal division of the segments of the apical initial or initials. But in many angiosperms the protoderm is *directly continuous* with the outermost tunica layer of the shoot apex (cf. Exercise III, p. 35). In roots, the surface

layer originates in a variety of ways from the apex and, according to Linsbauer (1930, p. 176), should be designated as the "*epiblem*" rather than as a true epidermis.

I. Structure of the Uniseriate Epidermis

In the majority of seed plants, the epidermis is an uniseriate layer of cells, the histology of which is extremely variable and often complex. Aside from the epidermal appendages or *trichomes*, which will be discussed later in this exercise, the common cell types found in this layer are (1) various kinds of secretory and crystalliferous *idioblasts*, (2) the *epidermal cells* proper, and (3) the *guard cells* and *subsidiary cells* of the *stomata*. The salient features of the two later categories will now be summarized:

1. *Epidermal cells.* These cells, although exhibiting wide variation in size, shape, and arrangement, are usually closely joined with one another, thus forming a compact sheet of cells without intercellular spaces. An exceptional condition occurs in certain petals that develop prominent intercellular spaces between the ordinary epidermal cells. Such spaces, however, are covered externally by a cuticle (cf. Eames and MacDaniels, 1947, pp. 358-359, Fig. 170). Epidermal cells are roughly "tabular" in form but, especially in the laminae of dicotyledonous foliage leaves, exhibit a characteristic *undulate contour* when seen in surface view. In stems and especially in the leaves of many monocotyledons, the epidermal cells tend to be conspicuously elongated in the direction of the long axis of the organ. The recent investigations of Matzke (1947) reveal that the foliar epidermal cells of *Aloe aristata* (on the basis of an analysis of 200 cells) exhibit an average of 10.885 faces. Such cells, viewed from the external surface, are, on the average, hexagonal. With the exception of roots and the submersed portions of certain aquatic plants, the *outer walls* of epidermal cells are covered by a more or less prominent layer of waxy material termed the *cuticle*. This layer is continuous, except for stomatal openings, and serves to restrict the loss of water from plant organs. The thickness of the cuticle is highly variable, in some organs being a hardly perceptible "film" whereas in other instances (e.g., fruits and many types of

leaves) it is extremely prominent (cf. Eames and MacDaniels, 1947, pp. 51-54). According to the recent review by Priestley (1943), in the shoot of angiosperms "a cuticle is present over the apex as a continuous layer of fatty substances overlying the cellulose walls of the outermost cells." The question, however, of the origin and method of excretion of the fatty substances that form the cuticle are yet to be fully resolved (cf. Priestley, 1943; Sifton, 1944, p. 107). The true *walls* of epidermal cells vary in their structure and especially in their chemical composition. Typically, the outer *tangential wall*, directly beneath the cuticle, is the thickest of all the walls, and the *inner tangential* wall the thinnest. Often the *radial walls* taper in thickness inwardly. Whether true secondary walls are commonly present in epidermal cells is uncertain. In many instances, however, the radial and inner tangential walls are provided with conspicuous *"pit-pairs."* According to Haberlandt (1914, p. 102) the innermost zone of the outer wall usually consists of "unaltered cellulose" and is followed externally by layers of wall substances that contain varying amounts of *cutin*. The work of Anderson (1934), however, has shown that in the leaf of *Clivia nobilis*, the thick *outer wall* of the epidermal cells shows "two distinct zones of cutinization." The outermost zone is devoid of cellulose or pectin while "the inner zone of cutinized wall consists of a series of cellulose lamellae separated by layers of pectic material, both of which are impregnated with cutin. The inner cutinized zone may be in direct contact with the protoplasm of the cell or may be separated from the protoplasm by a second zone of cellulose and pectic materials." It is clear from this work that a thorough study of the process of cutinization in epidermal walls of various plants is urgently needed (cf. Priestley, 1943 and Sifton, 1944).

A *protoplast* is normally retained in epidermal cells throughout their existence and a wide variety of ergastic materials such as anthocyanin pigments, tannins, oil, and crystals may occur. *Chloroplasts*, according to the résumé given by Linsbauer (1930, pp. 131-135), are by no means always absent but occur in the epidermal cells of a wide range of vascular plants. One of the most dynamic features of epidermal cells is their ability either in the course of "normal" organ development or in response to

wounding or chemical stimuli, to divide and to give rise to tissues or organs of the most varied character. "Adventitious" buds arising on leaves or stems commonly are formed from the "dedifferentiation" of epidermal cells (Buvat, 1944; Crooks, 1933; Link and Eggers, 1946; McVeigh, 1938; Naylor, 1940; Naylor and Johnson, 1937; Regel, 1876). In many plants, the phellogen arises by the tangential division of epidermal cells.

2. *Stomata.* The continuity of the epidermis, especially of foliage leaves and young stems, is interrupted by minute openings or pores termed *stomata.* Each stoma represents an intercellular space between a pair of highly specialized epidermal cells known as *guard cells.* As seen in surface view, guard cells are very frequently crescent-shaped with their concave surfaces adjacent to the slit-like pore. In contrast to ordinary epidermal cells, the walls of guard cells are uneven in thickness, often with ridge- or flange-like extensions at the edges of the pore. Furthermore, guard cells usually contain prominent *chloroplasts.* Since stomata play such an important role in the processes of respiration, photosynthesis, and transpiration, much attention has been devoted to the "mechanism" by which the stomata are "opened" and "closed." In general, changes in the width of the stoma are regulated by the relative degree of turgor in the guard cells, which in turn causes slight alterations in their shape. When the guard cells are turgid, the width of the pore is at a maximum; closing of the aperture occurs when the turgor of the cells decreases. A discussion of the variation in the construction of the walls of guard cells and of the physiological factors influencing the turgor movements of these cells, however, is beyond the scope of this book (cf. Haberlandt, 1914, pp. 445-477; Meyer and Anderson, 1939, Ch. XIII).

From a morphological standpoint, a number of types of stomata, based upon method of development, have been proposed and the relation of these types to problems of classification in the dicotyledons is summarized by Solereder (1908, pp. 1078-1081). The various types are based upon (a) the method of origin of the "mother cell" from which the pair of guard cells arises and (b) upon the presence or absence of *subsidiary cells.* The latter are cells that border directly upon the guard cells and differ from the

adjacent epidermal cells in size, shape, and arrangement. In the simplest condition, a protoderm cell divides anticlinally into two dissimilar cells, the smaller of which functions directly as the mother cell of the pair of guard cells, and subsidiary cells are not formed (examples: *Allium, Iris*). When subsidiary cells are present, they frequently arise from the division of the protoderm cells adjacent to the mother cell of the stoma. *Zea* and bamboo develop two subsidiary cells that lie at either side of the paired guard cells (Campbell, 1881, pp. 763-766 and Porterfield, 1937). In *Tradescantia*, two pairs of subsidiary cells are produced; these arise from the four protoderm cells that surround the mother cell of the stoma. According to Yarbrough (1934, pp. 474-475 and pl. 2) in *Bryophyllum calycinum*, the initial cell of the stoma first cuts out a spirally arranged series of subsidiary cells, its final division giving rise to a pair of guard cells. This distinctive type of stomatal development is designated in Solereder (1908, p. 1079) as the "Cruciferous Type" and seems characteristic not only of the Cruciferae and Crassulaceae but of other families as well. A more complete account of stomatal ontogeny lies beyond the scope of this book. For detailed information the student should consult De Bary (1884), Prat (1932), Solereder (1908), Strasburger (1866), and Tognini (1894).

II. Material for the Study of the Uniseriate Epidermis

1. The bulb-scale of the onion (*Allium Cepa*). Remove, with forceps, a small strip of epidermis from the outer or *abaxial* surface of the bulb-scale and mount it carefully in water. Under low magnification, note the rather orderly arrangement of the "rectangular" or tabular epidermal cells. *Stomata*, which may be abortive or "abnormal" in appearance, are occasionally present in this material. Under high magnification, it will be seen that the *radial walls* of the epidermal cells are provided with numerous small "*simple pits*." Because of the comparatively small radial depth of the epidermal cells, nuclei are readily seen. The cytoplasm is highly vacuolate and often is actively streaming or moving within many of the cells. Small greenish-yellow bodies, which may represent *elaioplasts* (oil-containing plastids), are also observable. To study the cuticle and the structure of the

walls of the epidermal cells, cut thin transections of the bulb-scale, and mount them in a .1% solution of neutral red.[1] If these sections are not overstained, the neutral red will be confined largely to the protoplasts and the walls will be clearly demarcated. Similar transections, when mounted in a solution of Sudan IV,[1] are particularly suitable for a study of the cuticle which will appear under high magnification as a brilliant red layer continuous over the outer walls of the epidermis.

2. The leaf blade of the geranium (*Pelargonium*) or the "German Ivy" (*Senecio mikanoides*). Remove small strips of the lower (*abaxial*) epidermis and mount some in water, others in a .1% solution of neutral red. Note especially the characteristic undulate contours of the epidermal cells and the numerous stomata. If the geranium epidermis is used, the relationship of the numerous *multicellular hairs* can be readily studied.

3. The leaf of *Iris* sp. Mount strips of the epidermis in water as well as in neutral red and examine carefully under low magnification, noting the regularly arranged stomata and the elongate form of the epidermal cells. Careful focusing will show that the guard cells with their stomata are slightly sunken beneath the surface and are overarched by the ends of the epidermal cells adjacent to them. Thin transections of the epidermis should also be secured and treated with neutral red and Sudan IV. These sections will reveal the thick cuticle and the prominently thickened outer tangential walls of the epidermal cells. "Simple pitpairs" are relatively abundant in the radial and inner tangential walls.

4. Prepared slides of transections of the leaf blades of lilac (*Syringa vulgaris*) and corn (*Zea Mays*). Transections cut by hand are often too thick to permit accurate examination of the structure of the walls of guard cells. For this reason, a supplementary study should be made of the guard cells as seen in thin, stained sections of the leaves of these plants.

5. Study strips of immature epidermis from the leaves of *Zea*, *Tradescantia* and *Sedum*, noting the various stages in development of guard cells and subsidiary cells. Mounting the

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[1] Cf. Appendix, p. 218.

strips in very dilute neutral red will greatly facilitate this investigation.

6. Examine demonstrations showing the origin and early development of adventitious buds from the epidermis. Tran- and longisections of portions of decapitated hypocotyls of *Linum* provide excellent material (for full details cf. Crooks, 1933 and Link and Eggers, 1946).

III. The Multiple Epidermis

In the leaves of certain angiosperms, particularly in various members of the families *Piperaceae*, *Begoniaceae* and *Moraceae*, some or all of the protodermal cells of the young lamina may divide tangentially, thus giving rise to a *multiseriate* or *multiple epidermis*. According to the résumé of the literature given by Linsbauer (1930, pp. 38-43), the first tangential divisions leading to the development of the multiple epidermis occur at a relatively late stage in leaf ontogeny; in *Ficus*, for example, as the leaf is beginning to expand within the bud. In *Mouriria Huberi* (Melastomataceae) the earliest tangential divisions in the adaxial protoderm occur while the lamina halves are still tightly revolute (Foster, 1947). The number of successive tangential divisions varies widely between different plants. In some, for example *Ficus elastica*, certain species of *Begonia* and *Mouriria Huberi*, the adaxial multiple epidermis is 3-4 cells in depth. At the opposite extreme may be cited the remarkable case of *Peperomia pereskiaefolia* in which the upper multiple epidermis of the leaf may attain a thickness of 14-15 layers and thus represent about 7 times the thickness of the entire mesophyll (for details cf. Pfitzer, 1872). During the final stages in development of a multiple epidermis, the outermost cells often divide predominantly in the anticlinal plane. As a result, at maturity it may appear as if a normal uniseriate epidermis, overlying a tissue of "colorless" cells, were present. It should be apparent from this fact that the proper concept of the multiple epidermis should be based upon its *origin* from the protoderm and not upon its adult histology or presumed function. This is important because much confusion still exists in the literature of physiological and systematic anatomy as a result of the failure to distinguish

clearly between the various types of hypodermal layers (originating independently of the protoderm) and the true multiple epidermis (cf. Linsbauer, 1930, pp. 38-39).

IV. Material for the Study of the Multiple Epidermis

1. The leaf of the rubber plant (*Ficus elastica*). As early as 1839 the German botanist Meyen (1839) described and figured the cystoliths in the multiple epidermis of *Ficus elastica*. His original description has been confirmed and extended by the recent investigations of Ajello (1941). During the early process of tangential division in the protoderm, certain cells fail to divide and instead become enormously distended inwardly, finally protruding into the underlying palisade parenchyma. These epidermal idioblasts are termed *lithocysts* and each one typically develops a *cystolith*. The latter at maturity consists of a stalk (morphologically, an invaginated growth of the outer wall of the lithocyst), the dilated end of which becomes incrusted with a dense aggregation of *calcium carbonate crystals*. Lithocysts occur in both the ad- and abaxial multiple epidermal layers in *Ficus elastica* but are particularly large and well developed on the upper side of the lamina (for a résumé of the structure and occurrence of epidermal cystoliths in dicotyledons cf. Linsbauer, 1930, pp. 53-58). *Obtain* a transection from a living leaf of *Ficus* and study it carefully under low and high magnification, noting the lithocysts and the well-developed "colorless" multiple epidermal layers. The outermost epidermal cells on each leaf surface are overlaid by a cuticle, are smaller than the underlying cells, and strikingly resemble a distinct uniseriate epidermis. Note particularly the *sunken stomata* in the lower or abaxial epidermis (cf. Meyen, 1839, taf. XI and De Bary, 1884, p. 104, Fig. 44). The *inner cells* of the multiple epidermis are relatively large and, because of their shape, their cellulose walls and the presence of intercellular spaces suggest resemblance to cortical parenchyma tissue. Notice that the cells are not aligned in radial rows because, during their formation, both anticlinal as well as periclinal divisions have occurred. A careful inspection of the walls of these cells under high magnification will reveal numerous "*simple pit-pairs.*" At intervals large sac-like *litho-*

cysts will be seen protruding into the adjacent palisade parenchyma. Each lithocyst, unless injured in sectioning the leaf, contains a *cystolith* with its knob-like end covered by a crystalline mass of calcium carbonate. Introduce a few drops of *hydrochloric acid* under the cover-glass and observe the rapid dissolution of the calcium carbonate. This is accompanied by the evolution of small bubbles of carbon dioxide.

2. The leaf of *Peperomia.* Study prepared stained transections of the leaf of *Peperomia,* noting the thick *adaxial* multiple epidermis and the uniseriate abaxial epidermis with stomata. Processed material usually shows the thin radial walls of the cells of the multiple epidermis buckled and distorted to varying degrees. For this reason, a comparative study of transections of fresh leaves should be made. These will also show the marked contrast in color between the mesophyll and the multiple epidermis. The leaf of *P. Sanderi,* available at most florists, or nurseries, is recommended for this study.

3. Study demonstrations that illustrate the origin and early ontogeny of the multiple epidermis. Serial paraffin sections of young and maturing laminae of the leaf of *Ficus elastica* provide graphic material and are highly recommended.

V. Trichomes

The term "trichome" is used in a very broad sense to designate collectively all the diversified unicellular and multicellular appendages that develop from epidermal cells. According to this strict usage, which was advocated by De Bary (1884, p. 58), "emergences" (e.g., the "prickles" on the stems of *Rosa* and *Ribes*) should be excluded from the concept of trichome because they are formed from cells derived both from the epidermis as well as from hypodermal tissue. The distinction between a "trichome" and an "emergence" is sometimes sharp as in those cases where a unicellular hair is situated on the apex of a well-defined emergence. (Cf. the "stinging hair" of *Urtica* depicted in Haberlandt, 1914, p. 130, and Eames and MacDaniels, 1947, p. 116, Fig. 56A.) But, as Netolitzky (1932, pp. 64-69) has shown, the *basal portion* of many multicellular "trichomes" is derived partly from the epidermis, partly from hypodermal cells. In

short, a rigid demarcation between "trichomes," "emergences," and intergradations between these structures cannot be drawn ontogenetically.

It should be clear from the above résumé that efforts to classify the varied types of trichomes have met with the same difficulties encountered in classifying internal cell types. Haberlandt (1914, p. 126), emphasizing the *functional aspects*, states that "obviously only those trichomes which assist the epidermis in its protective capacity can be regarded as epidermal appendages in an anatomico-physiological sense." Netolitzky (1932, pp. 7-10) reviews the different *morphological classifications*, which are based upon the form, number, and structure of the component cells of trichomes. He also presents a useful summary of the principal modes of development of unicellular and multicellular trichomes. It is evident from his résumé that trichomes furnish a rich field for morphogenetic investigations because of their great diversity and because their superficial position and relatively simple structure facilitate study with living material. Explorations in this direction should ultimately clarify the morphology of many trichomes that are exploited so frequently in taxonomic and genetical investigations. (Cannon, 1909; Heintzelman and Howard, 1948; Rollins, 1944; Solereder, 1908, pp. 1114-1130.) As a brief introduction to the comparative morphology of trichomes, four of the common types will now be described. Additional types are described in detail in the foregoing literature.

1. *Hairs.* In structure, hairs may be conveniently subdivided into (a) *unicellular types*, which in turn may be tubular or branched to varying degrees, and (b) *multicellular types*, which consist of a single row of cells (uniseriate) of several layers (e.g., the so-called "shag hairs," discussed by Netolitzky, 1932, pp. 66-67) or are branched often in a dendroid manner (cf. Eames and MacDaniels, 1947, p. 170, Fig. 80H). Multicellular hairs may be composed of essentially similar cells but in many cases terminate in a unicellular or multicellular *"gland."* Two general regions may be distinguished in a typical hair, viz.: (1) the *foot*, which is is the portion lying within the epidermal surface, and which is often different in form from the adjacent epidermal cells, and (2) the *body*, which is the portion extending away from the epi-

dermal surface. In the ontogeny of many kinds of multicellular hairs, the first division of the initial cell is periclinal and the body of the hair develops acropetally by cell division from the outer cell (cf. Netolitzky, 1932, pp. 56-61). Occasionally, a given epidermal surface develops only a single type of hair. More commonly, especially in leaves, varied types of hairs may occur intermingled on the same epidermal surface.

2. *Scales.* These distinctive trichomes consist of a discoid plate of cells and frequently are situated on a short stalk-like emergence. The ontogeny of the scale in *Shepherdia canadensis* has been investigated by Cooper (1932). It arises from a single initial cell which by repeated longitudinal and oblique divisions gives rise to a plate-like structure. According to Cooper, not all the cells composing the scale radiate from a common center. This condition results from the unequal and oblique divisions of certain of the primary cells early in the development of the scale (cf. also the development of the scale of *Hippophae rhamnoides* illustrated by Netolitzky, 1932, p. 62, Fig. 14).

3. *Colleters.* On many foliar organs, particularly on bud scales and stipules and on the foliage leaves of certain genera (e.g., *Aesculus, Rhododendron, Carya*) glandular trichomes occur. These structures were originally termed "colleters" by Hanstein (1868). Colleters consist of a short, often multicellular stalk bearing an expanded disc or knob of *secretory cells*. The characteristic sticky exudation found on certain foliar structures is secreted from colleters (cf. Foster, 1929, pp. 457-458).

4. *Water vesicles or bladders.* Trichomes of this type consist of greatly distended epidermal cells presumably of physiological importance as reservoirs for water (cf. Haberlandt, 1914, p. 116). In the so-called "Ice Plant" (*Mesembryanthemum crystallinum*), the water vesicles are so large and so numerous that the leaves and young stems appear to be covered with minute translucent beads of "ice."

VI. Material for the Study of Trichomes

1. *Hairs.* Obtain thin transections of the petiole of the geranium (*Pelargonium*) and, after mounting them in water, study carefully the structure of the unicellular and the multicellular

unbranched hairs. Note the relatively thick outer walls of the body of these hairs; actively streaming cytoplasm is usually evident in most of the cells. The foot, especially of the multicellular hair, consists of an enlarged bulbous cell, separated by a transverse wall from the body, and surrounded by a circular group of more or less elevated *subsidiary cells.* The true relationship of the subsidiary cells to the foot of the hair is clearly seen in thick transections of the petiole as well as in strips of epidermis removed from the lower surface of the lamina. Note that certain of the multicellular hairs are glandular, terminating in a single, large secretory cell filled with dense, yellowish-brown ergastic material.

Excellent material for a study of the structure of "stinging hairs" is found in the common nettle (*Urtica* spp.). Secure a transection of the living stem or petiole of this plant and mount it in water or dilute neutral red. Investigate the structure of the large unicellular stinging hairs, noting especially (1) the enlarged bulbous base surrounded by the cells of the pedestal-like emergence and (2) the swollen tip of the hair. When such a hair is touched, the apex is broken and a jagged point thus is produced which penetrates the skin much like a hypodermic needle. According to Haberlandt (1914, pp. 130-132), who has studied in detail the stinging hairs of *Urtica,* the substance released from the trichomes is not formic acid but rather an albuminoid substance, enzyme-like in nature.

Leaves of various members of the Malvaceae afford material for the study of *stellately* branched hairs. Each hair of this type consists of a number of radiating unicellular "branches" that have arisen from the subdivision of a single epidermal cell. Further illustrations of multicellular branched hairs are provided by the leaves of mullein (*Verbascum Thapsus*). *Scrape* a small amount of hairs from the leaf into dilute alcohol, carefully tease them apart with dissecting needles, and examine under low magnification. Note that these complex hairs are "dendroid" in form; i.e., each hair consists of a main "axis" (composed of a vertical series of cells) and whorls of radiating unicellular or bicellular "branches." Transections of very young mullein leaves are very instructive in showing various stages in the ontogeny of these

hairs. The leaf-blade of the sycamore or buttonball tree (*Platanus*) likewise will provide examples of dendroid multicellular hairs.

If time permits, study demonstrations showing the origin and early development of hairs. For this purpose, transections of very young capsules of cotton (*Gossypium*) are very instructive and serve to illustrate the ontogeny of the most important economic plant hair (cf. Anderson and Kerr, 1938). Transections of buds of various common plants (e.g., *Pelargonium*, *Nicotiana*) will serve to demonstrate the development of multicellular hairs of various types.

2. *Scales.* Scrape a small quantity of trichomes from the leaf of *Shepherdia* or *Elaeagnus* into a drop of dilute alcohol on a slide and examine under low magnification. In *Shepherdia* two extreme types of peltate trichomes will be seen, viz.: (1) *stellate hairs*, consisting of a delicate stalk bearing ten or more distinct radiating unicellular "branches," and (2) *scales*, which are gray-yellowish brown in color, lobate, and consist of a plate of many cells borne on a short emergence.

3. *Colleters.* The bud scales of the horse-chestnut (*Aesculus Hippocastanum*) provide excellent material for a study of typical colleters. Cut thin transections of the inner scales of a winter bud and mount them in water. Under low magnification note that the abaxial surface of the scale in particular is densely covered with colleters. Instructive views are provided if the surface of the inner bud scales is studied at high magnification with a binocular dissecting microscope. Additional or alternative material for a study of colleters is furnished by the foliar organs of various species of *Alnus*.

4. *Water vesicles.* Obtain several thin transections of the petiole of the "Ice Plant" (*Mesembryanthemum crystallinum*) and examine them in water under low magnification. The *adult* water vesicle appears as a large, clear hemispherical cell that projects outwardly from the general epidermal surface. Under high magnification, a nucleus, scanty cytoplasm and small plastids may be detectable in the bladders. If the concave adaxial surface of the petiole of immature leaves is examined, various stages in the origin and expansion of the water vesicle may be seen.

VII. Suggested Drawings and Notes

1. *The uniseriate epidermis.* Prepare carefully labeled drawings of epidermal strips of *Allium, Pelargonium* or *Senecio,* and *Iris.* To illustrate the varied arrangements of the subsidiary cells of stomata, draw small portions of young and mature epidermal strips from the leaves of *Zea, Tradescantia* and *Sedum.* Representation of the surface views of the epidermis should also be accompanied by drawings of transections, especially of the living material of *Allium* and *Iris.*

2. *The multiple epidermis.* Prepare drawings based upon a study of the transections of the lamina of *Ficus elastica* and *Peperomia.* Include, in the drawing of *Ficus,* a lithocyst with its cystolith.

3. Prepare drawings to illustrate the adult structure of the various types of trichomes studied. Also represent, by a series of drawings, the ontogeny of a hair, as illustrated by *Gossypium* or other available material.

4. Prepare a résumé of recent investigations of the development and structure of the cotton hair (cf. Anderson and Kerr, 1938; and Hayward 1938, pp. 443-448).

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Exercise VI

PARENCHYMA

The term "parenchyma" is used in a rather abstract or loose sense to designate a wide variety of living cells that occur in many different regions of the plant body. Parenchyma cells may appear in groups scattered among highly specialized conducting elements, as for example the cells of vascular rays and the vertical files of phloem and xylem parenchyma cells. Often, however, parenchyma cells form homogeneous or "simple" tissues which may constitute a large part of the softer regions of leaves, stems, roots, and fruits. From these illustrations it should be clearly evident that under the concept of "parenchyma" are included cells that differ markedly in their form, structure, position, origin, and functions. For this reason, "parenchyma" is merely a convenient and long-established anatomical category within which are included cell types not necessarily either homologous or analogous.

In an effort to characterize parenchyma tissue more precisely, it is commonly described as being composed of cells essentially isodiametric in form, with thin walls and active protoplasts. To appreciate the merits as well as the limitations of this set of characteristics, the following critique will be found useful.

I. General Features of Parenchyma

1. *Form of cells.* During the past two decades, a long series of intensive studies has been devoted to a geometrical analysis of the form of plant cells, with particular emphasis upon parenchymatous cells and the factors that determine their shape. Extensive discussions of the literature in this field are given in the recent papers of Lewis (1944) and Matzke (1946) and need not be considered in this book. As a result of an ingenious technique for isolating parenchyma cells (cf. Marvin and Matzke, 1939), together with the reconstruction method of Lewis (1944), it has been possible to make models of single cells or of cell groups. The statistical analysis of such cell models reveals that unspecialized parenchyma cells closely approach the form of

14-sided polyhedra or *tetrakaidecahedra* (cf. Matzke, 1946, p. 74, table 8). The forces that mould or determine this trend of parenchyma cells toward the form of *orthic tetrakaidecahedra* (bodies with 8 hexagonal and 6 quadrilateral faces) have not been fully explored but both compression (Marvin, 1939) and interfacial or surfaces forces (Matzke, 1946) are apparently operative during tissue differentiation. Many cells, however, classified under "parenchyma," diverge strikingly in their adult form from the comparatively regular cells investigated by Marvin, Matzke, and their associates. A spectacular example is provided by the remarkable *"stellate"* or *"armed" parenchyma cells* found in the pith of *Juncus*. These cells develop on the average 12 "arms" and are separated by large air spaces. Their ontogeny has been studied by Lewis (1925) and more recently by Geesteranus (1941) and various factors, such as the tension of the expanding pith and the turgor of the developing arms, are cited by the latter as operative in their development from unspecialized parenchymatous tissue.

2. *Structure and chemistry of the wall.* During the differentiation of the parenchyma cells in the cortex and pith of many stems and in the mesophyll of leaves, little or no appreciable increase in wall thickness occurs and a true secondary wall, distinct from the original primary wall, is not formed. In such cells, the thin primary wall seems to consist largely of cellulose. *Primary pit fields* are usually present but may be restricted to certain facets of the cell wall. In contrast, the cells of the *wood parenchyma* and *wood rays* in secondary xylem, usually develop conspicuously thickened, lignified walls. In the Abietoideae and in many woody dicotyledons, true secondary walls provided with pits are developed. But in the ray cells of the Taxodiaceae, Araucariaceae, Taxaceae, Podocarpaceae, Cupressaceae and Cephalotaxaceae no secondary wall is developed (Bailey and Faull, 1934). It may be questioned whether it would prove serviceable to "subdivide" parenchyma into two major classes based upon the presence or absence of secondary walls. It is nevertheless clear, however, that, in present usage, the term "parenchyma" embraces an exceptionally wide range of living cell types.

3. *The protoplast.* The retention of an *active protoplast* represents one of the most important characteristics of parenchyma cells. Indeed, because of this fact, parenchyma cells perform many of the most fundamental physiological processes, notably photosynthesis, food and water storage, secretion and excretion (Meyer, 1923; Netolitzky, 1935; Sperlich, 1939). Not infrequently, one or more of the above functions may be carried on in the same cell. Frequently, however, *parenchymatous idioblasts*, differing to varying degrees from their neighbors, function as cells that produce and accumulate oils and tannin or calcium oxalate (cf. Ex. I, p. 5-7). In addition to their important metabolic activities, however, parenchyma cells retain to an exceptional degree an ability to (a) resume growth and differentiate into sclereids of various types and (b) to "dedifferentiate" to the state of meristematic cells from which the most varied tissues (e.g., cork, callus) or structures (adventitious buds and roots) may arise (cf. Bloch, 1941, 1944; Buvat, 1944, 1945; Priestley and Swingle, 1929). It may be argued that the ease with which parenchyma cells can be induced to divide and to produce thereby new tissues and organ primordia is evidence of their "unspecialized" nature. But it is evident that many factors, hereditary as well as environmental, influence the reactivation of parenchyma and that our knowledge of the potentialities of such tissue is still in an exploratory phase (cf. especially Bloch, 1947).

II. Material for the Study of Parenchyma

At this time, suggested material for a study of typical, thin-walled parenchyma is described. Additional examples of parenchyma are given in later exercises in this book.

In the subepidermal region of young stems and in the mesophyll of leaves, the thin-walled parenchyma cells contain chloroplasts and perform the function of photosynthesis. *Obtain* a thin transverse section of the stem of *Begonia gracilis*, and, after mounting it in water, examine the preparation at low magnification. Notice that the cortex (i.e., the region between the epidermis and the cylinder of vascular bundles) and the pith are composed of large, thin-walled "isodiametric" cells. (*Note:*

Several layers of small collenchyma cells are found at the outer edge of the cortex and may be disregarded in this study.) Under low magnification observe that large, solitary *prismatic crystals* as well as *druses* occur in many of the parenchyma cells. Frequently, the form of the crystals is highly irregular. Under high magnification, the cytoplasm, vacuole, and small chloroplasts can be readily studied, especially if the sections are mounted in .1% solution of neutral red. Because of the large size of the cells, the nucleus is only seen occasionally in transections of the parenchyma tissue. Small *primary pit-fields* may be visible in certain walls of the cells. In order to understand the shape and proportions of the parenchyma cells, as well as the intercommunication between the prominent intercellular spaces, longisections of the stems should be studied under low and high magnification. In this plane of section, the parenchyma cells are arranged in vertical columns or files, as the result of their development from a typical rib meristem (cf. Ex. III, p. 31). An excellent wax-plate reconstruction of the comparable pith-parenchyma of *Eupatorium dubium* is given by Lewis (1944, p. 621, Fig. 2). Observe that in *Begonia* the intercellular space system in the longisection appears "black" because of the included air. This optical effect is of great assistance in distinguishing between walls and longitudinally extended air spaces.

To appreciate fully the polyhedral shape of parenchyma cells, examine *macerations*[1] of the pith tissue of *Begonia, Coleus* or *Eupatorium*. If this material is incompletely macerated, highly instructive three-dimensional views of connected series of cells may be obtained. Mount all material in dilute neutral red.

For a study of *stellate parenchyma* cells, examine tran- and longisections of the pith of *Juncus*, mounting them in neutral red solution. Very satisfactory sections of living stems may be secured with the aid of the CO_2-Freezing microtome, provided that the material is thoroughly aspirated before sectioning. Thick transections, which may be cut by hand, are highly instructive if viewed with a binocular dissecting microscope under moderate magnification.

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[1] For technique of macerating thin-walled parenchyma tissue, cf. Appendix p. 215-216.

III. Suggested Drawings and Notes

1. Prepare drawings, from both tran- and longisections, showing the structure of the parenchyma tissue in the cortex or pith of the *Begonia* stem. Include cells that show crystals, cytoplasm, and chloroplasts and that represent, in both planes drawn, the intercellular air spaces.

2. Prepare a drawing of a small portion of the pith of *Juncus*, illustrating the form and interrelationships of a group of stellate parenchyma cells as seen in transectional view.

3. Prepare a summary of the results of experiments with lead-shot and bubbles on the problem of cell shape determination in parenchyma (cf. Marvin, 1939 and Matzke, 1946).

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Exercise VII

COLLENCHYMA

The peripheral region of many stems, petioles, and ribs of leaf-blades is occupied by a more or less homogeneous tissue termed *collenchyma*. Frequently this tissue occurs as a continuous hypodermal cylinder, but, in some stems and petioles, discrete *strands of collenchyma*, laterally separated by parenchyma, may be present. Good illustrations of this strand-like development of collenchyma are found in the petioles of celery (Esau, 1936, Pls. 2 and 6) and *Heracleum sphondylium* (Majumdar, 1941, Pl. 1, Figs. 1 and 2). Within recent years, intensive studies devoted to the structure and development of collenchyma have extended considerably the classical concept of this tissue outlined by De Bary (1884, pp. 119-120).

I. Structure, Ontogeny, and Function of Collenchyma

1. *Morphological "types" of collenchyma.* The term "collenchyma" was first introduced into plant histology by Schleiden in 1839 to designate the thick-walled hypodermal tissue of cacti. As Müller (1890) has shown in a thorough historical review, the subsequent extension of this term occurred in two directions. From a *morphological standpoint*, collenchyma designated a tissue composed of living, usually elongated cells with walls conspicuously thickened *at the corners*, which occurs commonly beneath the epidermis in stems and petioles (cf. De Bary, 1884, pp. 119-120). From a *physiological standpoint*, Schwendener (1874) regarded collenchyma cells as a type of "stereide" or supporting cell, a concept later taken over by Haberlandt (1914, pp. 155-158). Although Müller (1890) recognized these morphological and functional aspects of collenchyma, his survey of the petioles of more than 400 angiosperms convinced him that the classical notion of collenchyma structure was too narrow. He proposed a classification of collenchyma into seven more or less distinct "forms," based primarily upon the distribution of thickened areas of the wall and their relation to intercellular

spaces. In the light of subsequent researches it appears that a sharp line cannot be drawn between a number of the forms of collenchyma recognized by Müller. Indeed, intergradations frequently occur in successive radial portions of the same zone of collenchyma. Majumdar (1941, pp. 39-40) in a critique of Müller's classification, accepts the following three types as inclusive of the commonest forms of collenchyma, viz.: (1) "angular collenchyma" (Müller's "Eckencollenchym"), the "classical" type in which the thickened areas of the wall represent vertical strips which are most conspicuously developed at the junctions between three or more cells. In this form of collenchyma, intercellular spaces are small or entirely wanting. Examples: *Solanum lycopersicum, Datura, Apium;* (2) "lacunate collenchyma" (Müller's "Luckencollenchym"), a highly contrasted type in which the wall thickenings are virtually restricted to those portions of a cell-group bordering upon well-developed air spaces. As Müller points out, this type in transectional view gives the illusion of a series of small, isolated, thick-walled cells diffused in a parenchymatous "matrix." Examples: Many members of Compositae especially strongly developed in *Petasites niveus:* (3) "plate collenchyma" (Müller's "Plattencollenchym") which consists of compactly arranged cells, the *tangential* walls of which are excessively thickened. This results in the appearance of more or less distinct successive tangential thickened plates or bands. Examples: *Astrantia, Eupatorium.* Majumdar (1941) regards the collenchyma he studied in *Heracleum* as falling within a separate category for which he has proposed the term "strand collenchyma."

2. *Structure and chemical composition of the wall.* Since collenchyma cells, during the normal ontogeny of the plant or in response to wounding, have the capacity for division and growth, the unevenly deposited wall should be regarded as a *primary wall* (cf. Ex. II, p. 11). Several recent investigations have been devoted to the structure and the nature of the organic constituents of this wall. Anderson (1927) working on the "angular collenchyma" of tomato, described the finely lamellated nature of the thickenings as the result of an alternation of cellulose-rich, pectic-poor, cellulose-rich layers. Majumdar (1941) found a com-

parable situation in the walls of the "strand collenchyma" of *Heracleum*. But in their studies of the "lacunate collenchyma" of *Petasites vulgaris L.* Preston and Duckworth (1946, p. 347) state: "It is not, however, by any means clear that the cellulose-rich cellulose-poor alternation of lamellae as found in other types actually occurs here at all." Their results, although not entirely conclusive, suggest that in *Petasites* (a) the cellulose is more evenly distributed throughout the wall thickenings and (b) that a segregation of pectic compounds into definable lamellae "is difficult to demonstrate." The relatively high percentage of pectic material in the cell walls of collenchyma is a typical feature and is apparently the basis for the high water content of the walls (for details cf. Esau, 1936, pp. 453-454, and Preston and Duckworth, 1946).

3. *Ontogeny*. According to Esau (1936, pp. 451-453) the origin of the collenchyma bundles in celery is traceable to the localized periclinal and anticlinal subdivision of ground meristem cells in the young petiole. As a result of repeated longitudinal divisions, procambial-like strands of elongated, thin-walled cells are produced and it is from such cells that the adult collenchyma is differentiated. At first the young collenchyma cells are relatively small in diameter, but, as the rate of cell division slows down, the cells expand and gradually acquire the characteristic angular wall thickenings. The origin and early development of the collenchyma in *Heracleum* closely resemble that in celery except that Majumdar (1941, pp. 26-31) regards a portion of the strand as derived from "prodesmogen cells" (i.e., cells that produce the radially adjacent vascular bundle). The *degree* to which *intercellular spaces* are maintained during collenchyma differentiation in the plants mentioned above is variable. In the case of celery, Esau (1936, p. 453) states that "the rapid succession of divisions in the young collenchyma strand causes a close packing of cells and loss of those intercellular spaces that were present among the initiating ground-meristem cells." She states further that "perhaps the deposition of wall material in the corners aids in closing intercellular spaces along with the packing of dividing cells." However, certain of the late-developing collenchyma strands in celery are initiated in more lacunate tissue

and intercellular spaces "are frequently maintained in the mature strand." According to Majumdar (1941) the obliteration of intercellular spaces occurs *before* wall thickening begins. He describes an early phase in collenchyma development in *Heracleum* in which the spaces are entirely filled with pectic substances. It seems clear that further studies on the origin and fate of intercellular spaces in various types of collenchyma tissue are definitely needed.

Longitudinal sections and macerations reveal that the mature cells of angular collenchyma are conspicuously elongated with oblique or tapered ends. One very long cell in *Heracleum* measured 2490 μ in length (Majumdar, 1941, p. 26). Very commonly, however, collenchyma cells become *septate* as a result of one or more transverse or oblique divisions of the original elongated mother cell (cf. Esau, 1936, Pl. 8, and Majumdar, 1941, pp. 31-32, Fig. 6).

4. *Function.* Collenchyma tissue provides considerable strength as well as elasticity to young stems and to leaves. Its *early appearance*, prior to the differentiation of sclerenchyma, suggests that it must possess considerable flexibility during the phase of elongation of an internode or of a petiole. In celery, Esau (1936) found by experiment that the collenchyma strand "is much stronger than the vascular tissue. The breaking load of collenchyma may be two to four times that of the entire vascular bundle or the bundle cap." In terms of the construction of the wall, Preston and Duckworth (1946, p. 350) attribute the strength and elasticity of collenchyma to the "longitudinal arrangements of the cellulose chains, giving a high ultimate tensile strength, while the non-crystalline component assures considerable extension without fracture, a feature with which the suggested greater angular dispersion of the cellulose in the innermost layer is also associated."

II. Material for the Study of Collenchyma

1. The petiole of *Datura* spp. provides excellent material for the study of the "angular" type of collenchyma. (*Note:* If *Datura* is not available, the stems of *Solanum lycopersicum*, *Cucurbita*, or *Begonia* are recommended.) *Prepare* with the

aid of a razor blade transections of the living petiole of *Datura* and study them in water-mounts under low and high magnification. The *epidermis* possesses thickened outer walls covered by a prominent cuticle; a protoplast and small scattered chloroplasts should be visible in many of the epidermal cells. Note the prominent *multicellular unbranched hairs* and study carefully the structure of their component cells. Beneath the epidermis occur six or more layers of typical *angular collenchyma cells*, the irregularly thickened walls of which exhibit a *pearly white lustre.* This white refractive appearance of the walls is apparently typical of all types of collenchyma when examined in the living state. The innermost layer of collenchyma cells in *Datura* lies in direct contact with the large-celled parenchyma of the petiole. In order to study critically the wall structure of the collenchyma, *obtain* thin trans- and longisections of the petiole cut with the aid of the CO_2-Freezing microtome. If such sections are mounted for study in dilute neutral red solution, the walls become brilliantly stained and the lamellations are often clearly revealed. Very dilute ruthenium red solution may also be employed to demonstrate the occurrence of pectin compounds in the wall. A study of thin transections under high magnification will reveal further (a) the occurrence of *intercellular spaces*, variable in size and distribution. Not infrequently rather conspicious tangentially elongated spaces will be found between the thick inner tangential walls of the epidermal cells and the subjacent collenchyma cells; and (b) the existence of a protoplast and small *chloroplasts* in many of the collenchyma cells. To understand thoroughly the structure of angular collenchyma, *study* under high magnification the thin longisections, noting the bar-like character of the thickened areas of the walls. *Primary pit-fields* are often evident in various regions of the wall and the intercellular spaces may appear as more or less conspicious vertical lacunae in various parts of the tissue. Note especially that many of the collenchyma cells are *septate*, with very thin transverse or oblique division walls.

2. The petiole of celery furnishes readily available material illustrating collenchyma tissue developed in distinct strands or bundles. Transections cut with a razor blade should be mounted

in both water and dilute neutral red solution. This study will be facilitated if Esau's (1936) paper is available in the laboratory.

3. The petioles of various dicotyledons will afford material for a comparative study of the "lacunate type" of collenchyma. Although this type is most strikingly developed in *Petasites niveus*, according to Müller (1890, p. 162), such genera as *Rudbeckia, Senecio, Dahlia,* and *Salvia* may be used.

III. Suggested Drawings and Notes

1. Draw on a large scale a *sector* of the transection of the petiole of *Datura* (or of the substitute material previously listed) about 5 to 6 cells in width, extending from the epidermis to the cortical parenchyma tissue. Label carefully all important structures.

2. Prepare a drawing of a small portion of the collenchyma tissue as seen in longisectional view. Show clearly the distribution of the thickened areas of the wall, the primary pit-fields and the intercellular spaces. Fill in the contents of a single collenchyma cell.

3. Prepare a diagram to illustrate the position of the collenchyma strands in the celery petiole. The position of the oil ducts and the vascular bundles should also be indicated. *Draw* a small portion of the collenchyma as seen in transectional view.

4. Summarize, in the form of laboratory notes, the evidence discussed by Esau (1936) which shows that the walls of collenchyma cells are rich in water.

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Exercise VIII

SCLERENCHYMA: SCLEREIDS

The term "sclerenchyma" was originally proposed by Mettenius (1865) to designate all thick-walled parenchymatous and prosenchymatous cells exclusive of the bast fibers of the vascular system. As Foster (1944) has recently shown, this effort to demarcate two categories of "mechanical" elements on a purely topographical basis was adopted by a number of histologists in the 19th century. However, soon after the appearance of Mettenius' paper two rather divergent trends of thought developed with reference to the designation and classification of thick-walled supporting cells. On the one hand, the concept of sclerenchyma was broadened morphologically to include the fibers of the vascular system as well as the varied types of extra-fascicular thick-walled elements. The latter were designated, depending upon their form, as "stone cells" and "fibers" (cf. De Bary, 1884; Hayward, 1938). On the other hand, a more strictly physiological viewpoint was developed by Schwendener (1874) who termed mechanically significant cells, such as collenchyma, bast fibers, and libriform fibers, "stereids." According to Schwendener, stereids collectively constitute the "stereome" or mechanical tissue system of the plant. In his classification, the extra-fascicular "sclerenchyma cells" of Mettenius were excluded, presumably because their strengthening function had not been demonstrated. The ideas of Tschirch (1885a, 1885b, 1889) reflect the influence of both Mettenius and Schwendener. Tschirch maintained that many extra-fascicular sclerenchyma cells are mechanically important in the life of the plant (Tschirch, 1885b). For all such elements that are non-prosenchymatous in form he proposed the term "sclereid." Haberlandt (1914, pp. 158-161) eventually included sclereids as one of the cell-categories under the "mechanical tissue system."

From the above historical résumé it is evident that the problem of differentiating and classifying the varied types of thick-

walled cells in plants is far from being solved. Whether the physiological concept of "stereome" or the essentially morphological concept of "sclerenchyma" is adopted, many difficulties arise. In the first place, the problem is aggravated by the very frequent occurrence of cell types, intermediate in form and structure, between "typical" sclerenchyma cells and other cell categories. A good example is provided by the secondary xylem of many seed plants in which intergradations are found between libriform fibers (in which the "mechanical function" is emphasized) and tracheids (in which the "conducting function" is emphasized). A thorough discussion of this problem is given by Bailey (1936). A further example is provided by the difficulty of demarcating fibers from sclereids. In the leaves of *Camellia* (Foster, 1944) and *Trochodendron* (Foster, 1945a), *both* fiber-shaped and stellately branched sclerenchyma cells occur and are morphologically connected by transition forms. Intergradations are also common between parenchyma cells and more or less isodiametric "stone cells." Lastly it must be emphasized that recent investigations show that the most varied types of branched or fiber-like sclereids may be restricted, in the leaves of certain dicotyledons, to the ends of the veinlets (Foster, 1946, 1947). Such "terminal sclereids" morphologically could be regarded as part of the vascular system of the leaf and hence would be excluded, on a topographical basis, from the category of sclerenchyma as conceived by Mettenius (1865). It seems very evident, therefore, that a more exact classification of the highly variable types of sclerenchymatous elements that occur in vascular plants must await further intensive as well as extensive investigation. In the treatment of sclerenchyma adopted for laboratory instruction in this book, sclereids and fibers are presented separately with the full recognition that such a procedure is largely one of convenience in handling a large and extremely complex problem.

I. Sclereids

1. *General features and classification.* Sclerenchyma cells of this type occur in a wide range of positions in the plant body, frequently as hard masses or nests of cells embedded in softer

tissues. Some regions or tissues of the plant may be composed almost exclusively of sclereids, for example, the hard shells or "pits" of many fruits and the tough skins of seeds. Very commonly, however, sclereids develop as *idioblasts*, i.e., cells that are usually strikingly different in form, size, and in the thickness of their secondary wall from neighboring tissue elements. Idioblastic sclereids fluctuate widely in form, and bizarre types are found in the leaves of certain dicotyledons such as *Camellia*, *Trochodendron* and *Mouriria* (Foster, 1944, 1945a, 1946, 1947), as well as in the stem of *Pseudotsuga* (Sterling, 1947) and the aerial root of *Monstera* (Bloch, 1946). In *Monstera*, the sclereids are long, slender and "hair-like" in form and have been termed "trichosclereids" by Bloch. As Foster (1944) has pointed out in detail, a rich terminology developed during the 19th century for the varied forms of idioblastic sclereids. As a guide to the student in reading, especially in European texts and journals, a few of the more common terms will be mentioned here. In much of the German literature, as, for example, in Solereder's (1908) classical "Systematic Anatomy of the Dicotyledons," idioblastic sclereids are designated as "*spicular cells*." The French histologists, such as van Tieghem (1891), early adopted the term "*sclerité*" for the same type of element. Because the arms or processes of many idioblastic sclereids lie freely within large intercellular spaces, such cells have been termed "*internal hairs*," "*ground-tissue hairs*" or "*trichoblasts*." In the case of the foliar sclereids of *Camellia, all* of the above special terms have been applied at various times by different writers. Clearly there is need for clarification and standardization of descriptive terms. The word "sclereid" is brief and etymologically clear and has much therefore to commend it (cf. Eames and MacDaniels, 1947, pp. 88-89). Doubtless as investigation proceeds, it may prove desirable to set up a series of sclereid categories, based on form development and wall structure. An attempt in this direction was made by Tschirch (1889, pp. 301-302) who proposed four main categories of sclereids according to the form of the cell, viz.: (1) *Brachysclereids*, or "stone cells," which are roughly isodiametric in form and which occur commonly in the pith, phloem, cortex, and "bark" of stems and in the fleshy portions

of many fruits, e.g., *Pyrus* and *Cydonia;* (2) *Macrosclereids,* or "rod-cells," which are columnar in form and often constitute an outer palisade-like layer in seed coats, e.g., in the family Leguminosae (here they are also referred to as "Malpighian Cells"); (3) *Osteosclereids,* or "prop-cells," which are likewise somewhat columnar in form but are dilated, lobed, or ramified at each end (such sclereids occur in seed coats and in the leaves of a number of dicotyledons); and (4) *Astrosclereids,* which are ramified to varying degrees, often in a rather regular stellate manner. Sclereids of this type are conspicuous features of the leaves of many seed plants. Despite the rather arbitrary nature of Tschirch's classification, it possesses considerable utility for purposes of compact histological descriptions. Ultimately a more natural classification of sclereids will recognize fully *the range of form-variation within a species* or even an organ and will utilize additional characters derived from ontogeny and from wall structure (Bailey and Nast, 1944). In this connection the remarkable sclerenchyma cells of *Welwitchia* (Bower, 1881; Chamberlain, 1935), *Araucaria* (Seward, 1906), many of the Nymphaceae (Conard, 1905, pp. 54-94), and of *Schisandra* and *Kadsura* (Bailey and Nast, 1948) should prove distinctive types because all of them exhibit *prominent crystals* embedded in their walls.

2. *Ontogeny and structure.* In the light of recent investigations, it is clear that the ontogeny of sclereids, particularly the idioblastic types, offers many points of great histogenetic interest. The following resumé is merely intended as a guide to the problems and to the relevant literature.

Typical brachysclereids develop by the "secondary sclerosis" of parenchyma cells (cf. De Bary, 1884, pp. 539-541). This process involves the centripetal development of a massive lignified secondary wall which is often conspiciously laminated and which may be provided with *ramiform pits;* the latter are morphologically simple pits with coalescent canal-like cavities. Frequently there is little change in the size and shape of a cell that experiences secondary sclerosis, and hence the mature brachysclereid closely resembles in form the parenchyma cell from which it originated. The physiological factors that induce

the sclerification of parenchyma cells are obscure, but often brachysclereids originate near wound tissue, suggesting some type of disturbed physiological reaction (Bloch, 1944). In the case of maturing bark of woody stems and roots, large numbers of parenchymatous elements become transformed into sclereids, suggesting that sclerification in this case may be an aspect of *tissue senescence.* A further interesting example of the origin of brachysclereids by secondary sclerosis is exhibited by the development in many stems of the so-called "composite cylinder" composed of both sclereids and bast fibers. In this case, an originally continuous cylinder of bast fibers becomes ruptured at various points as a result of the increase in diameter of the stem. Neighboring parenchyma cells then intrude into the gaps, divide to varying degrees, and finally become transformed into brachysclereids, thus "repairing" the broken cylinder (for details cf. Tschirch, 1885a, p. 323, et seq.; and Haberlandt, 1914, p. 159).

Very recently detailed investigations have been made of the ontogeny of the branched type of idioblastic sclereid in the leaves of *Camellia japonica, Trochodendron aralioides* and *Mouriria Huberi* (Foster, 1944, 1945b, 1947), in the aerial roots of *Monstera deliciosa* (Bloch, 1946) and in the stem of *Pseudotsuga taxifolia* (Sterling, 1947). Two fundamental histogenetic problems are illustrated by the development of the sclereids in these plants, viz.: (1) *The position of the sclereid-initials in the developing tissues.* In all the above cases, the sclereid originates from a small, thin-walled uninucleate initial. In *Camellia, Trochodendron* and *Pseudotsuga* these initials are diffused, apparently "at random," throughout the parenchymatous tissue. But in *Monstera,* according to Bloch (1946), the sclereid initials are formed in a more definable "pattern," originating from late divisions in the vertical files of differentiating cortical parenchyma cells. The situation in the lamina of *Mouriria Huberi* is an even more striking example of an orderly pattern of development of sclereid initials, since they are predominantly restricted to the ends of the developing veinlets. The factors that control the random or ordered position of sclereid initials are unknown, but a more complete understanding of them would illuminate the entire problem of idioblasts within plant tissues (cf. Bloch, 1947). (2) *The*

method of enlargement and ramification of the sclereid. Originating as a small cell, the sclereid soon begins to enlarge and to branch, ultimately attaining a grotesque and bizarre form (Foster, 1944, 1945b, 1947). In some instances, as in the leaf of *Trochodendron* and the aerial root of *Monstera*, the developing sclereid abuts upon prominent air spaces into which may of the arms extend and ramify. But the sclereid processes, to varying degrees, also grow *between* the walls of neighboring tissue elements. Space does not allow a full discussion of the various theories that have been proposed, such as "gliding growth," "intrusive growth" and "interposition growth," to account for intercellular adjustments of elongating and branching cells during histogenesis. (For critical discussion and literature, cf. Sinnott and Bloch, 1939; Foster, 1944, 1947 and Sterling, 1947.) The present evidence, however, favors the idea that the developing sclereid branches grow between the primary walls of tissue elements in their path much as a pollen tube pushes its way through the tissues of the stigma and the style or as the tip of a fusiform cambial initial intrudes between its neighbors. Whether the middle lamella is partially or wholly destroyed during such intercellular growth remains to be investigated. Furthermore, the effects of "intrusive" or "interposition" growth upon plasmodesmata is still problematical, although there is some indirect evidence that new protoplasmic connections may be produced as the sclereid acquires new cell contacts during its primary development. After a branched sclereid has completed its intercellular development, a thick lignified secondary wall is developed. The pitting fluctuates from relatively sparse, as in *Trochodendron, Mouriria* and *Monstera,* to extremely abundant, as in the petiolar sclereids of *Camellia.*

Although sclereids are commonly regarded as "dead" cells when fully mature (Eames and MacDaniels, 1947, p. 89), this generalization seems incorrect in the light of several careful investigations. Puchinger (1923), for example, found that a protoplast may be retained in various types of sclereids as long as the organ in which they occur remains alive and functional. According to her observations, sclereids may retain their proto- ... stems for 2-4 years, in certain "evergreen" leaves for

1-5 years, and in the endocarp of certain fruits for 1-1½ months. The "stone cells" (i.e., brachysclereids) of the fruits of quince (*Cydonia*) and pear (*Pyrus*) provide an additional example of the retention of a protoplast. Alexandrov and Djaparidze (1927) contend that in this material it is possible to demonstrate, by staining with safranin and methyl green, the presence of nuclei in the mature branchysclereids. These investigators further maintain that, during the ripening of the fruit of *Cydonia*, the sclereids experience a process of "delignification" consisting of the reduction in thickness of the wall, the disappearance of lignin, and the obliteration of the ramiform pits. This reversible change suggests enzymatic activity on the part of the protoplasm within the stone cells. Crist and Batjer (1931), in their detailed study of the stone cells of *Pyrus*, state that the transformation of a lignified wall to one of cellulose reported by Alexandrov and Djaparidze for *Cydonia* does not occur in Kieffer and Bartlett pears ". . . without exception, the downward trend of the cellulose curve is strictly parallel to that of lignin and each one of the two is parallel to the ligno-cellulose trend." Further study is obviously needed to determine more precisely the chemical relations between the sclereids and the neighboring parenchyma tissue during fruit ripening.

(3) *Function.* Sclereids, because of their thick, strong walls, undoubtedly are mechanically effective in many plant structures, producing a hard texture which, in the case of seed coats, endocarps of fruits, etc., may be biologically advantageous. Haberlandt (1914, p. 158) states that brachysclereids "serve to increase the incompressibility of the bark, their action may be compared to that of the sand which a mason uses to increase the tenacity of his mortar, or to that of the powdered glass which is added to gutta-percha in order to render it less compressible." But in many organs, the functional significance, if any, of the sclereids is problematical, as for example in the case of the "nests" of brachysclereids in the fleshy portion of pear fruits. It has been suggested that phylogenetically they may represent the remains of a former continuous shell of stone cells. Likewise, the physiological role of idioblastic sclereids in the lamina of the leaf is open to question. Although they doubtless in such cases are

concerned in producing a stiff or coriaceous texture (cf. Tschirch, 1885b), it remains to be proved whether this purely, passive, mechanical function is biologically significant.

II. Material for the Study of Sclereids

The great diversity in form, position, and structure of sclereids can be most clearly understood by a survey of selected organs in various seed plants. As far as possible, widely distributed and easily available plants have been selected, supplemented by recommendations for the study of herbarium material to illustrate certain important sclereid types.

1. *Sclereids in stems.* The stem of the "wax-plant" (*Hoya carnosa*), which is widely cultivated as a conservatory plant, provides excellent material for a study of sclereids developed in the *pith*. Transections of the young stem will show a great variety of stages in the *secondary sclerosis* of pith-parenchyma cells, including the development of a laminated secondary wall and the origin of ramiform pits. Further illustrations of sclereids in stems are readily provided by cutting thin hand-sections of the fruit stalk (morphologically a stem) of *Pyrus* and mounting them directly in phloroglucinol-hydrochloric acid.[1] Add a cover-glass and examine the sections under both low and high magnification. The edge of the section consists of several layers of *cork*, the innermost cells of which are in contact with the *phellogen* or cork cambium. Internal to the phellogen occurs the *cortex*, composed of thick-walled parenchyma tissue in which are embedded clustered as well as idioblastic *brachysclereids*. Progressing inwards, there next occurs a cylinder of *vascular bundles*. Each of these bundles consists of an external cap of fibers (which usually stain less deeply than the sclereids), a strand of *phloem*, and a strand of *xylem*. The *pith* of the fruit stalk is composed both of parenchyma cells as well as of groups of brachysclereids.

2. *Sclereids in leaves.* An adequate study of the form, structure, and distribution of idioblastic sclereids in leaves requires the combined use of sections, macerations, and cleared leaf-sectors. The relatively simple procedures involved in the two latter techniques are described on pp. 215-217 of the Appendix.

[1] Cf. Appendix p. 217.

The choice of material will depend upon the plants available and the time apportioned for this exercise. The suggestions given below are made on the basis of successful classroom experience and include plants that should be available to most teachers.

(a) *Camellia.* Cut thin transections of the fresh petiole of the leaf and mount them directly in phloroglucinol-hydrochloric acid. Under low and high magnification, study the form and pits of the fiber-shaped and variously branched sclereids situated in the subepidermal "collenchyma" tissue. Similar sclereids are also found near the xylem of the large, crescentic vascular bundle. Macerations of the petiolar sclereids should also be studied in order to appreciate fully the polymorphic character of the sclereids and the abundant development of spicules (cf. Foster, 1944 for details and illustrations).

(b) *Osmanthus.* This genus contains several widely cultivated species and is recommended for a study of the form and distribution of sclereids in the lamina. Examine a cleared portion of the lamina of *O. aquifolium*, noting the abundant small, columnar sclereids, ramified at each end, which are dispersed as idioblasts in the mesophyll. The sclereids in the lamina of *O. fragrans* are larger and more profusely and irregularly branched.

(c) *Monstera deliciosa.* This widely cultivated aroid provides excellent material for a study of idioblastic sclereids in a monocotyledon. Portions of the cleared lamina are very instructive for demonstrating the abundance and the distribution of the slender "trichosclereids" (cf. Bloch's 1946 study of the trichosclereids in the air-root of *Monstera*).

(d) *Nymphaea* sp. The large, ramified sclereids occurring in the leaves of various water lilies are a classical example of so-called "internal hairs" or "trichoblasts" (cf. p. 95 of this exercise). Obtain a stained transection of the lamina of *Nymphaea*, noting the large sclereids, the arms and processes of which extend both into the prominent air chambers of the mesophyll as well as within the palisade parenchyma. Observe particularly that small angular crystals are embedded within the sclereid walls (for an extended description of sclereids in water lilies, cf. the monograph by Connard, 1905, pp. 54-94).

(e) *Hamamelis virginiana.* This widely distributed plant

provides useful material for a study of "terminal sclereids" in the lamina. An unpublished survey by the author has revealed considerable fluctation in the relative abundance of foliar sclereids in this species and preliminary study of local specimens should be made to assure suitable classroom material. If herbarium material is available, sclereids appear under low magnification as small, raised protuberances on the lower surface of the lamina. Portions of the cleared lamina should be stained in safranin. Such preparations reveal the presence of small, ramified sclereids at the terminations of the veinlets.

(f) *Pseudotsuga taxifolia.* The large, ramified idioblastic sclereids which occur in the needle-leaves of this conifer provide interesting material for comparison with angiosperm sclereids. Examine entire needles that have been cleared, noting the form of the sclereids and their orientation within the leaf. The literature treating of gymnosperm sclereids is discussed by Sterling (1947).

3. *Sclereids in fruits.* Obtain a small fragment of the fruit of pear and mount it in water under a cover-glass. Embedded in the thin-walled parenchymatous tissue will be found small clusters of *brachysclereids* that often appear yellowish-brown in color. Examining one of these groups of sclereids under high magnification, note their greatly thickened walls and the characteristic *ramiform pits.* Gentle tapping on the cover-glass with a needle or scalpel will frequently help to flatten the irregular mass of sclereids and thus facilitate observation. Following this preliminary study, remove the cover-glass, drain off the excess water, and apply a drop of phloroglucinol-hydrochloric acid. The brilliant red color assumed by the walls of the sclereids often aids in the study of the ramiform pits.

This study of the mature brachysclereids of *Pyrus* should be accompanied, if possible, by an examination of microtomed sections of very small fruits. Such preparations show clearly that the clusters of stone cells originate from parenchymatous elements by the process of secondary sclerosis.

4. *Sclereids in seeds.* The seed coats of bean (*Phaseolus*) or pea (*Pisum*) provide material for the study of typical "macrosclereids." Obtain a small amount of partially macerated bean

or pea testa and examine it under low magnification, noting the groups of small, tightly packed columnar macrosclereids. Observe that the lumen of each sclereid is widest near the base of the cell, being reduced to a narrow, virtually occluded channel above. This study of macerated material should be supplemented, if possible, by an examination of microtomed sections of young and mature seed coats. (For a detailed investigation of the structure and ontogeny of the macrosclereids and osteosclereids in the integument of *Pisum*, cf. Reeve, 1946a and 1946b. The literature on seed coat histology is also cited and discussed.)

III. Suggested Drawings and Notes

1. Prepare drawings to show the form and the ramiform pits of a small group of mature brachysclereids in the fruit of the pear. Draw also several stages in the *development* of the sclereids from the study of prepared transections of young fruits.

2. Make a diagrammatic drawing of the transection of the fruit stalk of the pear, showing and labeling all the important tissues and regions. Summarize in the form of laboratory notes the positions and probable mechanical significance of the brachysclereids and fibers in this organ. For comparative purposes, prepare drawings to illustrate the origin of brachysclereids in the pith of the stem of *Hoya carnosa* from the secondary sclerosis of parenchyma cells.

3. Study transections of fresh petioles of *Camellia japonica* stained with phloroglucinol-hydrochloric acid. Prepare (a) a diagrammatic drawing showing the positions of the sclereids with reference to other tissues and (b) drawings of several different forms of petiolar sclereids. If possible, supplement this record by drawings made from macerations. Examine, under low and high magnification, portions of the *cleared laminae* of various angiosperm leaves, noting the distribution and form of the sclereids. Prepare diagrams and drawings to illustrate your observations. (*Note:* For choice of material, refer to pp. 101 and 102.)

4. Draw a series of connected macrosclereids from the testa of bean or pea seeds. If possible, examine both macerations as well as prepared sections.

5. Prepare a brief written report on the problem of the intercellular development of branched sclereids. For interpretations and literature, refer to the papers of Foster (1944, 1945b, 1947), Bloch (1946), and Sterling (1947).

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Exercise IX

SCLERENCHYMA: FIBERS

I. Fibers

1. *General features.* In the strict histological sense, the term "fiber" designates a widely distributed type of cell in the plant body distinguished by its elongated form, thick secondary wall, and reduced pitting. Fibers are classical examples of "prosenchyma" cells, i.e., elements that differ from "isodiametric" parenchyma cells by their considerable length and by the overlapping of their acuminate ends. Commercially, the term "fiber" is employed in a loose sense to indicate either unicellular hairs (e.g., cotton, kapok) or multicellular strands of tissue. The latter have recently been subdivided by Dewey (1943, p. 1) into (a) "hard or leaf fibers," illustrated by the multicellular strands of fiber cells, often accompanied by vascular tissue, derived from such monocotyledons as henequen, sisal and abacá, and (b) "soft or bast fibers," illustrated by the soft flexible multicellular strands of fibers derived from the stems of such dicotyledonous plants as hemp, flax, jute, and ramie. In the present book, the word "fiber" will be used only in the strict histological sense defined above.

Fibers are the most important type of mechanical cell that occurs in vascular plants and their great tensile strength, flexibility, and elasticity serve to enable plant organs successfully to withstand a variety of strains and tensions resulting from the action of gravity, wind, etc. (cf. Haberlandt, 1914, pp. 161-187). Many plants are cultivated exclusively for the strong, economically important fibers that they produce. Among the more important of such textile plants may be mentioned *Agave fourcroydes* and *Agave sisalana,* known respectively in commerce as "henequen" and "sisal"; *Musa textilis,* or so-called "Manila hemp," which according to Dewey (1943, p. 50) should be known as "abacá"; *Cannabis sativa,* or true "hemp"; *Corchorus* spp., commercially termed "jute"; *Boehmeria nivea,* which is "ramie";

and *Linum usitatissimum*, or "flax," from which linen is derived. According to Hayward (1938, p. 371) "there is evidence that flax was grown during the Stone Age" and that the annual form of *Linum usitatissimum* "has been grown in Mesopotamia for at least 4000 years."

Because of the considerable economic importance of fibers, a very extensive literature has developed. The limited scope of this book precludes any effort to discuss in detail the many involved problems of wall structure and methods of development of fibers. Instead, a brief résumé is given now of the salient features of fibers and is intended as an introduction to the subject and a guide to the literature.

2. *The problem of classifying fibers.* Like sclereids, fibers are widely distributed in the plant body, occurring either as idioblasts (e.g., the leaflets of many cycads) or more commonly in the form of strands, a reticulum, or as a continuous cylinder. Fibers are particularly well developed in the vascular system, where they are distributed in a great variety of patterns in the phloem and xylem. Indeed as much as 50% of the wood of many angiosperms may consist of fibers. But fibers are also well developed in the "fundamental tissue system" of many plants where they form massive subepidermal cylinders or distinct bundle-like strands. Extra-vascular fibers are particularly well developed in the stems and leaves of numerous monocotyledons where they form, together with the parenchyma and the vascular bundles, a nearly "endless" series of structural patterns (cf. Schwendener, 1874; Haberlandt, 1914, pp. 169-184; and Meeuse, 1938). As is true of sclereids, intensive research on the ontogeny and the structure of fibers is necessary before a reasonable morphological classification becomes feasible (cf. Bailey, 1936; Esau, 1943c, pp. 583-585). Purely for convenience, a simple *topographical classification* will be adopted in this book according to which fibers may be divided into two principal types, viz.: (1) *Wood*, or intraxylary fibers, and (2) *Bast*, or extraxylary fibers.[1] On this basis, "bast fibers" would include the fibers that are morphologically a part of the phloem as well as the fibers that invest vascu-

[1] Cf. Haberlandt (1914, pp. 720–721) for a critical résumé of the history and uses of the term "bast" in plant anatomy.

lar bundles or occur in the fundamental tissue system of the stem, root, and leaf (cf. Esau, 1943b, p. 351). Although the inclusion of such a wide variety of fibers under the category of "bast fibers" is admittedly arbitrary, it is preferable, in the light of present knowledge, to the attempts to subdivide extraxylary fibers into a series of topographical classes such as "cortical fibers," "phloem fibers," and "pericyclic fibers." A classification of this sort needs ontogenetic evidence to support it but on this very point our information is still relatively meager. The best illustration of the problems involved is provided by recent investigations on the ontogeny of so-called "pericyclic fibers." As Esau (1943a, p. 195) points out, van Tieghem (1882) devised the term "pericycle" to designate the cylinder of sclerenchyma and the subjacent parenchyma that occurs external to the vascular bundles in the stem of *Cucurbita*. With the notable exception of several European investigators, whose ontogenetic studies are reviewed by Esau (1943a, 1943c), the concept of "pericycle" in stems primarily developed with reference to a topographical region situated between the endodermis and the primary phloem. Since fibers very commonly occur in this region, they have been widely designated in anatomical texts as "pericyclic fibers." Esau (1938b, 1943c), however, has shown that the so-called "pericyclic fibers" in *Nicotiana* and *Linum* are derived from the procambium and hence morphologically are a part of the primary phloem. Kundu (1941, 1942) reached a similar conclusion on the basis of a developmental study of the fibers of hemp, jute, *Crotalaria* and *Hibiscus*. Additional comparative ontogenetic studies are urgently needed in order to clarify further (1) the morphology of the varied types of extraxylary fibers and (2) the classical concept of pericycle. The present status of the problem, with reference to the stem, is admirably summarized by Esau (1943c, p. 583) who states: "The above review justifies the generalization that the strands of fibers located in mature stems, on the periphery of the stele adjacent to the phloem, arise in the phloem. These fibers form eventually homogeneous strands of tissue, because the sieve-tubes and companion cells of this area are completely obliterated before the fibers mature."

3. *Form and length of fibers.* In contrast to the parenchym-

atous or branched form of sclereids, fibers typically are slender, markedly elongated cells with acute or acuminate ends. Occasionally one or both ends of bast and wood fibers may be lobed or forked. In short bast fibers, the ratio of the diameter of the cell to its length may average 1:10 or 1:20, whereas in extreme cases (e.g., in the family Urticaceae) the ratio may reach 1:4000. These figures, taken from De Bary (1884, p. 131), emphasize the fact that certain bast fibers may represent some of the longest cells in higher plants. According to Hayward's (1938, p. 241) discussion of the literature, hemp (*Cannabis sativa*) fibers vary in length from 1-10 cm. In flax (*Linum*) the length of the fibers ranges from 2.5 mm. to 1.2 cm. Apparently the longest bast fibers that have been accurately measured occur in the stem of *Boehmeria nivea,* a member of the Urticaceae. In this species, Aldaba (1927) succeeded, by means of a special maceration technique, in isolating individual fibers, the five longest of which measured "respectively 400, 500, 520, 540 and 550 mm."

4. *Structure and chemistry of the cell wall.* Mature fibers possess a well-defined secondary wall, often so thick that the cell lumen may be almost or entirely occluded at various points. Because of the great economic importance of fibers and because their massive secondary walls, which are often highly lignified, provide exceptionally favorable material for physical-chemical studies, a voluminous literature has developed. It lies beyond the scope of this book to attempt to review or to evaluate the investigations that have been made. As an indication, however, of the complexity of the problems and as a guide to the literature, the student is referred to the work of Bailey and Kerr (1935). These investigators have discovered a wide range of structural pattern in the fiber-tracheids and libriform fibers of various woody dicotyledons. They found that "the visible structural pattern of the cellulosic matrix varies greatly in form and texture, not only in different plants, but also in homologous cells of the same plant, and even in different parts of the same cell." As our knowledge develops of the way in which the secondary wall of the fiber is formed by the protoplast, this structural diversity may become clarified. In this connection, much ontogenetic work remains to be done on the *pits* of fibers. These structures, in

typical wood fibers, may lack a border and frequently possess extended slit-like inner apertures (cf. Exercise II, p. 19). In many bast fibers, the pits appear simple; they fluctuate in abundance but are often very sparsely developed.

5. *Ontogeny of fibers.* In some instances, fibers originate from fusiform cambial initials and experience little or no subsequent elongation as they mature. But in many cases fibers arise from initial cells that are very short as compared with the length of the fully developed elements. An impressive example of this fact is furnished by Aldaba's (1927) work on fiber development in *Boehmeria nivea.* In this plant, the fiber initials "are approximately 20 microns in length" and "the increase in the longitudinal dimension of the longer bast fibers is of the order of 2,500,000 per cent, but the process of elongation is gradual and extends over a number of months." The investigations of Aldaba (1927) and Anderson (1927) on flax fibers have revealed many peculiarities, but our knowledge of fiber development in other forms is still meager. It is apparent that in certain bast fibers, the upper ends of the elements remain delicate and active during the phase of cell elongation. Whether the necessary adjustment between such greatly extending cells and their neighbors is achieved by "sliding" or "interposition growth" (Schoch-Bodmer, 1945) or by "symplastic growth" (Meeuse, 1942) is not yet entirely clear. Esau (1943c, p. 583), for example, states that the short length of the secondary—as contrasted with the primary phloem fibers in *Cannabis* "seem to indicate that the elongation of the primary bast fibers in harmony with other tissues plays a very important role in determining the ultimate length of these fibers."

The behavior of the protoplast during the growth and differentiation of certain types of fibers offers a number of points of interest. Esau (1938a) has shown that, during the elongation of the primary phloem fibers in tobacco, the protoplasts become multinucleate as a result of repeated mitotic divisions of the nucleus. Cell plates, however, do not form at the ends of the successive nuclear divisions and "the spindle fibers are less persistent than in ordinary division figures." At the final stages in fiber ontogeny, usually after secondary walls have developed, the

nuclei appear to fuse or clump and in nearly mature fibers "the nuclear material frequently occurs as one large degenerating mass." The bast fibers of *Linum* also exhibit a multinucleate phase during differentiation (Esau, 1943c). The physiological significance of this multinucleate condition in young phloem fibers is quite obscure. Haberlandt (1914, p. 154) who has observed a multinucleate protoplast in the bast fibers of *Linum* and certain members of the Leguminosae maintains that "the presence of several nuclei appears advantageous when the very considerable length of many bast-cells and their active growth in length and thickness are taken into account." In certain types of fibers, however, mitosis is followed by cytokinesis, resulting in a chambered or *septate fiber*. This condition has been observed and described by Vestal and Vestal (1940) in a study of the septate fiber-tracheids of *Hypericum Androsaemum*. In this species, the fiber-tracheid retains its protoplast after the thick secondary wall has been laid down. Mitosis may then occur in such a cell, the division figure being oriented parallel to the long axis of the cell. Cell plate formation then occurs in the normal manner and a thin transverse septum is formed across the lumen, intersecting the inner edge of the secondary wall of the "mother cell." Because of the delicacy of this septum it was not possible to determine whether it is "formed only of intercellular cement substance or whether it consists of the intercellular substance and two adjacent primary walls."

II. Material for the Study of Fibers

1. *Bast fibers.* Examine, under low and high magnification, macerated bark of the twig of the basswood or linden tree (*Tilia* sp.). The numerous prosenchymatous cells present are bast fibers from the phloem region. Select an unbroken fiber and study carefully its form and wall structure. Note especially the channel-like lumen and the small *pits*. To appreciate fully the arrangement and mechanical significance of the fibers in the phloem of *Tilia*, strip off a small portion of the bark from a twig or young branch and scrape off the outer tissues (i.e., epidermis, periderm and cortex) with a scalpel. Then mount the fibrous tissue that has been exposed in water and examine it under low

magnification, using a binocular microscope and note the *closely joined strands* of grayish-white bast fibers. In order to determine the degree of lignification of the secondary walls, treat the fibrous network with phloroglucinol-hydrochloric acid.

Permanent, stained preparations of various stems and leaves should also be studied. Transections of the stem of *Linum* are especially instructive because of the groups of large bast fibers found next to the innermost cells of the cortex (cf. Esau, 1943c, p. 580, Fig. 1). The *leaves* of many monocotyledons likewise provide striking material for a study of bast fibers. Secure a prepared transection of a portion of the leaf of *Agave* spp., noting the massive caps of bast fibers that accompany the vascular bundles. If material is available, hand-sections of the leaf of *Phormium tenax* are excellent to illustrate one of the many "patterns" of bast fibers found in monocotyledons (cf. Haberlandt, 1914, pp. 178-179 and Fig. 63A).

2. *Wood fibers.* (a) *Libriform fibers.* The fibers present in the secondary xylem of woody dicotyledons often show massively thickened secondary walls provided with scattered and rather small pits. In such cells, termed "libriform" because of their structural resemblance to phloem fibers, the lumen varies in width and may be entirely occluded at certain points. Study the form, structure, and pitting of the libriform fibers in stained longisections as well as macerations of the wood of oak (*Quercus* spp.).

(b) *Septate fibers.* This type of fiber, which may also develop in the phloem (Esau, 1948), is characterized by the subdivision of the lumen into a series of compartments separated from each other by transverse walls or septa. The work of Vestal and Vestal (1940) already discussed in this exercise has shown that in *Hypericum*, the septa of the fiber-tracheids arise, *after* the formation of the lateral secondary wall, as a result of repeated mitoses accompanied by cytokinesis. Doubtless the septate fibers in other genera of the angiosperms pursue a similar ontogeny. Secure a small amount of macerated xylem from the stem of the grape vine (*Vitis* sp.) and study under high magnification the form and structure of the numerous septate fibers. Note that the septa in these cells extend to the inner edge of the second-

ary wall but are independent of the compound middle lamella of the "mother cell." If the septa are examined with the aid of an oil-immersion lens, it is apparent that they have a laminated as well as a "pitted" structure. The lateral secondary wall of the fibers of *Vitis* are abundantly pitted and frequently the remains of the protoplasm may be evident within the various compartments of the cell.

III. Suggested Drawings and Notes

1. Prepare careful drawings based on a study of macerated material to illustrate the form, character of the lumen (i.e., occluded, septate, broad), and the type and distribution of pits of the bast fibers of *Tilia,* the libriform wood fibers of *Quercus,* and the septate wood fibers of *Vitis.*

2. Diagram the arrangement of the strands of bast fibers as seen in freshly scraped portions of the bark of the stem of *Tilia.* This study should be accompanied by an examination of the bast fibers in prepared tran- and tangential longisections.

3. Prepare a drawing to show the structure and position of the bast fibers of *Linum* as seen in the transection of the stem. For comparison, prepare diagrams and drawings illustrating the arrangement of bast fibers with reference to the vascular tissues in the leaf of *Agave* spp. or *Phormium tenax.*

4. Write a brief résumé of the commercial process of "retting" fibers in hemp or flax. (For information and additional literature, cf. Hayward, 1938, pp. 242, 244-245, 409-410 and Dewey, 1943, pp. 66-69.)

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Exercise X

TRACHEIDS AND VESSEL ELEMENTS

One of the fundamental characters of the anatomy of the sporophyte of the Tracheophyta is the presence of a well-defined conductive or vascular system. Nägeli (1858, p. 9) in his classical investigations of this system, introduced the terms "xylem" and "phloem" to designate its two component tissues. In the most primitive vascular plants (i.e., the Psilopsida) the xylem and phloem are relatively simple histologically, but in higher plants, such as the angiosperms, these tissues are composed of a wide variety of cell types differing from one another in form, wall structure, content, and function. Despite their inclusive connotation, the terms "xylem" and "phloem" have proved convenient descriptive categories and have been widely adopted by most anatomists. While the functions of storage and support are performed to varying degrees by both the phloem and the xylem, these tissues are significant first of all in the transportation of water and solutes between root and shoot. This conductive function is possible not only because of the arrangement and structural characteristics of the cells themselves but also because the vascular tissues throughout the plant body form a continuous, interconnected system. This continuity of the vascular system is maintained in general throughout the ontogeny of the plant, new increments being added during development by the activity of shoot and root apices and, in plants with secondary growth, by the vascular cambium. The present exercise is devoted to an introductory study of the xylem, with particular reference to the structure evolution and ontogeny of its most definitive elements, viz.: the *tracheid* and the *vessel element*. For convenience in general discussions of xylem structure in this book, tracheids and vessel elements will be designated collectively as "tracheary elements." In the exercise dealing with the comparative anatomy of the stem, leaf, and root, additional information regarding xylem and directions for studying it will be given.

I. Structure and Morphology of Tracheids and Vessel Elements

Sanio (1863, p. 113), in a truly classical paper on wood histology, introduced the term "tracheid" and analysed the morphological similarities and differences between this cell type and the vessel element.

Following his pioneering investigations, many intensive as well as extensive studies have been made with the resulting accumulation of a vast body of literature. Indeed, probably no other cell types in the vegetative body of vascular plants have been studied so minutely from a comparative point of view as the tracheids and vessel elements. The basic distinction between these two types of cells consists primarily of the fact that the tracheid is an *imperforate* cell whereas the end-walls of vessel elements are provided with openings, termed *perforations*. Vessel elements usually arise from the procambium or from the vascular cambium as more or less discrete series of superposed or reticulately interconnected cells. The term "vessel" is used to designate collectively such a series of interconnected cells. In many cases, the vessels of secondary xylem are vertical tubes, the length of which varies from a few centimeters to several feet or more. The terminal elements of vessels end "blindly" without perforations. A brief summary of the salient features of tracheids and vessel elements will now be given.

1. *The tracheid.* From a phylogenetic standpoint, the tracheid is usually regarded as an original cell type of the xylem. In the ancient Psilophytales the xylem of the protostele appears to have consisted exclusively of tracheids, many of which were provided with ring-like or spiral thickenings (cf. Arnold, 1947, pp. 66-78). Modern vascular plants exhibit widely varying degrees of histological complexity in the xylem (including, in the highest types, vessels, parenchymatous cells and fibers) but tracheids are present in all groups including the angiosperms. As will be pointed out in detail later, the tracheid is used in phylogenetic argument as the starting point for the evolutionary changes which culminated in the origin of vessels. From a structural viewpoint, a mature tracheid is usually a conspicuously elongated cell, devoid of a protoplast, with moderately thick walls

and in transectional view is angular or polyhedral in form. As Frost (1930b) has emphasized, typical tracheids lack well-defined end-walls. The *secondary wall* of tracheids is developed in a wide variety of patterns, which have been studied in considerable detail. In *primary xylem* (i.e., the xylem originating from the procambium), the tracheids are provided with secondary wall thickenings in the form of rings (*annular thickenings*); one or more spiral bands (*spiral thickenings*); a series of interconnected bars (*scalariform thickenings*); a network of secondary wall material (*reticulate thickenings*) or else they are *pitted*. Similar secondary wall patterns also occur in the vessel elements of the primary xylem. A more detailed discussion of these wall patterns in tracheary elements will be given later in this exercise. In *secondary xylem* (i.e., the xylem produced by the activity of the vascular cambium) the lateral walls of the tracheids are provided either with transversely elongated bordered pits (*scalariform pitting*), as in many ferns and certain angiosperm groups, or with *circular* or *oval bordered pits*. The comprehensive surveys made by Bailey (1944a) and his associates clearly indicate that scalariform pitting is primitive in dicotyledons and that from it has evolved the *opposite* and *alternate* arrangements of small circular or oval bordered pits so characteristic of tracheary elements in many flowering plants. A curious additional structural feature of the tracheids (and many vessel elements) in seed plants is the superposition of a series of helical bands upon the pitted lateral walls. Good examples are provided by the tracheids of *Taxus* and *Pseudotsuga* and by the so-called "vascular tracheids" in the late wood of certain dicotyledons (cf. Brown and Panshin, 1940, p. 63, Fig. 12 and pp. 182-183, Fig. 35b).

2. *Vessel elements.* Voluminous data, secured by a wide series of comparative studies, have established the fact that vessel elements originated phylogenetically from tracheids. Bailey (1944a), in a recent comprehensive summary of the most important work on this problem, has emphasized that the evidence points to an *independent origin* of vessels in the five following groups of vascular plants: (1) *Selaginellales*, (2) Filicales, (3) Gnetales, (4) monocotyledons, and (5) dicotyledons. Before reviewing briefly the salient features in the evolutionary de-

velopment of vessel elements, a brief description of their structural characteristics is essential.

As stated previously, vessel elements are fundamentally distinguished from tracheids by the presence of *perforations*, which typically occur in the well-defined end-walls of the cells. For convenience in description, "the area of the wall (originally imperforate) involved in the coalescence of two members of a vessel is designated as the *perforation plate*" (cf. Glossary of Terms Used in Describing Woods, p. 7). In the most primitive type of vessel element, a more or less sharply defined *scalariform perforation plate* is present on each of the inclined end-walls. This type of plate consists of a series of transversely oriented slit-like openings (produced by the dissolution of pit-membranes) separated by bars of wall substance. As Frost (1930b) has shown in detail, there has been a general evolutionary trend in dicotyledons resulting in (a) the elimination of *borders* from the bars between the openings and (b) the reduction in number and the increase in width of the openings. In what is clearly the advanced type of vessel element, the inclined or the strictly transverse end-walls are provided with large oval or circular *simple perforation plates*. Excellent evidence, derived both from comparative surveys as well as from ontogenetic sequences, indicates that the simple type of perforation has developed from the scalariform through the gradual disappearance of the transverse bars (cf. especially Frost, 1930b, p. 201, Fig. 1). A third type of perforation plate, which probably is a specialization of the scalariform condition, is termed a *reticulate perforation plate*. It typically develops in vessel elements with transverse end-walls and appears as a network of anastomosing bars surrounding numerous small perforated areas (cf. Smith, 1935 and Kundu, 1942). Frost (1930a, p. 91) regards this type of perforation plate as "an anamolous oddity resulting from the breaking down of vestigial scalariform perforations in scalariform-porous woods." The *lateral walls* of vessel elements exhibit the same range of structural patterns found in tracheids. In the case of the vessel elements in the secondary xylem of dicotyledons, Frost (1931) has determined that scalariform bordered intervessel pitting is primitive and has given rise to opposite and finally to alternate pitting.

Because of the detailed treatment of the problem of *vessel-evolution* given by Frost (1930a, 1930b, 1931), Bliss (1939), Cheadle (1942, 1943a, 1943b, 1944) and Bailey (1944a), it will only be necessary in this exercise to emphasize certain of the most significant inferences. In the first place, it is now clear that, although perforations in all cases originated from the dissolution of the pit-membranes of tracheids, two unrelated trends of origin occurred in vascular plants. In the Gnetales, the perforations in the vessel elements of the secondary xylem arose from the disappearance of the membranes of the *circular bordered pits* of tracheids (Thompson, 1918; Jeffrey, 1917; Bailey 1944a). On the other hand, the perforations in the vessel elements of *Selaginella, Pteridium* and the angiosperms *initially* developed from the loss of the membranes of the *scalariform bordered pits* situated at the ends of the tracheid-like cells. The investigations of Bailey (1944a) and his associates furthermore show that in the dicotyledons vessel elements first appeared in the *secondary xylem,* from which point their evolutionary development has worked back into the primary xylem. Indeed, in many dicotyledons, the earliest formed portion of the primary xylem, i.e., the *protoxylem,* may be entirely devoid of vessels. In other words, in a given case the primary xylem may conspiciously lag behind the secondary xylem with reference to the evolutionary specialization of tracheary elements. The situation in monocotyledons, which on the whole lack secondary xylem, has been clarified very recently by the extensive investigations of Cheadle. Using the same statistical and correlation methods earlier employed by Frost, Cheadle found that in the *primary xylem* of monocotyledons, the primitive vessel element resembles that in the dicotyledons, i.e., it is a long tracheid-shaped cell with scalariform perforations. From this type, as in dicotyledons, the more highly evolved shorter vessel element with transverse end-walls and simple perforations has developed (1943a). According to Cheadle (1944) vessels phylogenetically originated in the *late metaxylem* (i.e., the last formed portion of the primary xylem) and have worked back in their subsequent development into the protoxylem. His survey (1943b) of the *distribution of vessels* in the various organs of monocotyledons revealed the probability that vessels first appeared in the root. In many monocotyledons

they are restricted to that organ. In others, vessel development has spread from the root upwards, to varying degrees, into the shoot system.

In concluding this account of vessel evolution, attention must be directed to the remarkable situation presented in three ranalian families, viz.: the Trochodendraceae, Tetracentraceae, and Winteraceae (Bailey, 1944b; Bailey and Nast, 1945). The members of these families are distinguished from the majority of investigated dicotyledons by the absence of vessels in *both* primary and secondary xylem. On the contrary, tracheids, provided with scalariform-pitting as well as with circular bordered pits, are the sole type of tracheary element present. Bailey (1944a, p. 421) states that "the vessel members in the less specialized types of dicotyledonous secondary xylem closely resemble the scalariform, bordered-pitted tracheids of these vesselless representatives of the Ranales." According to Bailey (1944b, p. 98), there is no possibility of these plants having developed vessels and subsequently having lost them, because of the primitive character of their cambia and xylem structure. Although *Trochodendron Tetracentron* and the members of the Winteraceae have attained the angiospermic level in their flowers, their wood exhibits a conspicuous evolutionary lag and, in the Winteraceae is most comparable with the secondary xylem of Pteridospermae and the Bennettitales (Bailey, 1944b, p. 99). It is entirely possible, as comparative studies progress, that other examples of primitively vesselless woods in angiosperms will be discovered and will shed additional light on the evolutionary history of tracheids and vessel elements. (Cf. the vesselless xylem of *Amborella* described by Bailey and Swamy, Jour. Arnold Arboretum 29:245-254, 1948.)

II. Ontogeny of Tracheids and Vessel Elements

1. *The formation of perforations in vessel elements.* The characteristic dissolution of more or less extensive portions of the end-walls of vessel elements has been noted by many investigators (cf. the historical review given by Esau and Hewitt, 1940, pp 229-231), and modern investigations on vessel ontogeny are centered on two important points, viz.: (1) the exact *time in vessel differentiation* when the break-down of the end-walls oc-

curs, and (2) the *physical and chemical nature* of the portion of the end-walls that is ultimately removed. The studies of Esau (1936) and of Esau and Hewitt (1940) have shed considerable light on both of these matters with reference to the formation of *simple perforations* in the vessel elements of a series of herbaceous angiosperms. Their observations first of all reveal that the end-walls remain *intact* until the vessel element has attained its full size and has laid down secondary thickening on its lateral walls and usually on the *peripheral region* of the end-walls. This fact was established even for the huge "drum-shaped" vessel elements of *Cucurbita* that expand enormously in diameter during ontogeny (cf. Esau and Hewitt, 1940, p. 242, pl. 2). Furthermore, in such a plant as celery, perforations develop progressively from one end of the vessel to the other; according to Esau (1936, p. 482) "stages from an intact end-wall to its complete absence may be found in one section of a vessel in which disintegration of end-walls is in progress." With regard to the second point noted above, the portion of the end-wall that breaks down *is entirely devoid of secondary thickening.* On the contrary, the perforation results from the dissolution of a discrete region of the primary walls and the middle lamella between two superimposed vessel elements. An investigation of the optical and chemical composition of the end-wall between vessel elements prior to its dissolution showed it to consist of two cellulose primary walls flanking the isotropic intercellular substance. No positive test for the presence of lignin could be obtained (Esau and Hewitt, 1940, pp. 233-235). In a number of the plants studied by Esau and Hewitt, the discrete area of the end-wall that later becomes perforated is conspicuously thickened and in sectional view is lenticular or "torus-like" in shape. Apparently very little intensive study has been made in recent years of the details of perforation-formation in the vessels of *woody plants.* Bailey (1944a, p. 423) states that in the helically thickened vessel elements of the primary xylem of woody dicotyledons "oblong perforations are formed by the dissolution of the primary wall between the more or less transversely oriented bars." According to Eames and MacDaniels (1947, p. 99, legend describing Fig. 49) in *Robinia Pseudo-Acacia,* a well-developed secondary wall is present on that portion of the end-wall of the vessel element

which ultimately, through disintegration, becomes the perforation. This statement is in direct opposition to the conclusions of Esau and Hewitt derived from a study of various hebaceous plants and indicates the need for further comparative investigations on vessel ontogeny in the angiosperms.

2. *Secondary wall patterns in the tracheary elements of primary xylem.* When the progressive development of the primary xylem from the procambium is studied in longisectional views, it is apparent that the *successive tracheary elements* often differ conspicuously from one another with reference to the pattern of the secondary wall. In many plants, the earliest formed tracheary elements (classified collectively as the *protoxylem*) are provided with either annular or spiral thickenings; the latter consist of one or more delicate bands of varying pitch. Later developed tracheary elements tend to exhibit more extensive developments of their secondary walls in the form of scalariform bars, a network, or conspicuous pitting. Very frequently, a given tracheary element may combine two or more of these types of wall sculpture. Such transitional cells were early recognized by anatomists and were termed "vasa mixta" (De Bary, 1884, p. 156). Despite the great histological and physiological interest of these fluctuating wall patterns, comparatively little attention has been given to their ontogeny. Several recent studies, however, seem to confirm the early work of Crüger (1855). He found, in a study of young living tracheary elements, that actively streaming bands of dense parietal cytoplasm correspond in position to the future position of the rings or bars of secondary thickening. Barkley's (1927) investigations of the tracheary elements of *Trichosanthes* lend support to Crüger's statements. She found that in killed and fixed material, "the spiral vessel of the protoxylem in its early stages has bands of peripheral cytoplasm which precede the spiral markings and have the same arrangement, and become the basis of the lignified spiral. The position of the cytoplasmic bands is determined by rows of vascuoles in the cytoplasm immediately preceding and during the formation of the cytoplasmic bands." In a similar way, the annular and scalariform types of thickening are predetermined by the pattern of vacuolation in the cytoplasm. Entirely com-

parable results were obtained very recently by Sinnott and Bloch (1945) in their study of the origin of tracheary elements from parenchyma cells during the regeneration of vascular strands in *Coleus*. According to them, "bands of densely granular cytoplasm can readily be observed in stained preparations as the first visible steps in the transformation of a pith cell into a ringed or reticulate xylem element." The investigations of Majumdar (1940, 1941) on the development of the protoxylem vessels of *Heracleum* have raised an additional point of interest. In contrast to other investigators, Majumdar contends that the deposition of secondary bands is preceded by the development of "lenticular thickenings" *of the primary wall*. On these "bases" or projections the secondary thickenings are ultimately deposited by corresponding bands of cytoplasm. According to Majumdar, the primary "bases" are unlignified and consist of both pectin and cellulose. It seems evident from these recent scattered investigations that the entire problem of the cytological aspects of secondary wall patterns in the tracheids of primary xylem demands wide comparative investigation.

The "causal aspects" of patterns in the secondary walls of tracheary elements are still obscure. Since cells with annular or spiral thickenings are usually differentiated during the phase of organ elongation, it is possible that these patterns are in some way related to rapid cell elongation. Evidence in favor of such an hypothesis has recently been secured by experimental procedures (e.g., use of X-rays and regulation of light) that affect growth in length of an organ (Smith and Kersten, 1942; Goodwin, 1942). These investigations show that, when elongation is retarded or inhibited, the development of annular and spiral elements was correspondingly retarded or inhibited, and the development of pitted elements favored.

3. *The problem of defining primary and secondary xylem.*

(a) *Protoxylem and metaxylem.* As Esau (1943a, pp. 188-194) has pointed out in a detailed and critical review, the original distinction between protoxylem and metaxylem was based upon *the relative time of appearance* of these tissues in ontogeny. *Protoxylem,* a term introduced by Russow (1872), served to designate the xylem elements that arise *first* in vascular differen-

tiation and therefore establish *the pattern of future differentiation of the primary xylem.* The term *metaxylem* gradually became used to designate the remainder of the primary xylem. In an effort to draw a morphological boundary between protoxylem and metaxylem, the types of wall sculpturing of tracheary elements were widely exploited. According to this viewpoint (cf. for example "Glossary of Terms Used in Describing Woods," p. 2; Eames and MacDaniels, 1947, p. 133), protoxylem consists of tracheary elements with annular or spiral thickenings; metaxylem of tracheary elements with reticulate and pitted secondary walls. In this kind of morphological classification, scalariform elements represent either a "transition" state between the two tissues or are included under protoxylem (cf. Foster, 1942, p. 84). It is clear, however, from Esau's review and from her own ontogenetic studies, that the types of wall sculpturing in tracheary elements do not provide reliable, consistent criteria for the separation of protoxylem from metaxylem. Indeed, in some plants "the sculpture of secondary walls of the metaxylem may vary from spiral to pitted." On the contrary, Esau advocates a complete reinterpretation of the concept of proto- and metaxylem based on ontogenetic considerations. From this viewpoint, protoxylem is conceived as that earliest-formed portion of the primary xylem laid down while an organ "is undergoing its major elongation." Protoxylem tracheary elements are very often excessively stretched and even ruptured, after their differentiation, in elongating leaf traces and stem internodes. In contrast, Esau regards the metaxylem as that portion of the primary xylem which matures very largely *after* organ elongation has ceased. Esau's recommendations are thus based fundamentally upon a dynamic conception of primary xylem development and should point the way to a more profitable approach to a very confused problem in anatomy.

(*b*) *Primary and secondary xylem.* In practice, it is very difficult to distinguish the boundaries between the primary and secondary vascular tissues, especially in leaves and stems. The protoxylem, in the sense used above, marks the "pole" of differentiation in steles and vascular bundles and can usually be identified by its position in relation to the later-formed portion

of the entire xylem system. But great difficulty arises from the attempt to establish the boundary between the outermost portion of the "metaxylem" (at least in stems and leaves) and the subjacent "secondary xylem." Much of the confusion in interpretation is produced by the incorrect assumption, widely adopted in many texts, that cell arrangement in the primary xylem is "irregular" or "disorderly" as contrasted with usual radial-seriation of cells in the secondary xylem. This viewpoint, in turn, springs from the erroneous idea that cell divisions in the procambium, from which the primary xylem originates, are irregular. As Esau (1943a, pp. 168-177; 1943b, pp. 350-353) has shown in detail, the plane of longitudinal divisions in young procambial tissue *may* be strictly tangential (i.e., periclinal) and hence *all* the primary xylem and primary phloem elements may exhibit radial alignment. It is, therefore, very evident that the arrangement of tracheary elements does not provide a consistent basis for demarcating primary and secondary vascular tissue. A good example is furnished by the radial alignment of the tracheary elements in both the primary and secondary xylem of the vascular bundle of *Trifolium* (cf. Eames and MacDaniels, 1947, p. 308. Fig. 140-A).

Apart from the question of cell arrangement, an *approximate boundary* between primary and secondary xylem can often be drawn by utilizing the structural differences between the respective meristems that produce these tissues. Thus in woody dicotyledons and in gymnosperms, the vascular cambium usually consists of two definable types of initials, viz.: ray initials and fusiform initials (cf. Exercise III, p. 39). In contrast the procambium is relatively "homogenous" in cellular structure (Esau, 1943a, pp. 175-177). However, very little is known at present regarding the nature of the "transition" in type of growth from a typical procambium to the method of cell formation in the vascular cambium (cf. Sterling, 1946). At any event, the author entirely agrees with Esau (1943a, p. 177) that procambium and cambium should not be regarded as two sharply distinct meristems but rather "as two developmental stages of the same meristem." In conclusion, brief mention must be made of an additional criterion that may prove helpful in making an approximate bound-

ary between primary and secondary xylem. This concerns the apparent difference in *average length of the tracheary elements* in the two tissues. Bailey (1944a, pp. 426-427) has summarized this point as follows: "There is an abrupt and conspicuous decrease in length of tracheary cells in passing from the last-formed primary xylem to the adjacent first-formed secondary xylem. This zone of unconformity between the primary and secondary bodies is indicative of a significant developmental hiatus and may prove to be the most reliable means of differentiating the outer boundary of the primary xylem in the stems of higher gymnosperms and angiosperms."

III. Material for the Study of Tracheids and Vessel Elements

1. *Morphology.* To understand thoroughly the points of similarity and of difference between tracheids and vessel elements, a reasonably broad laboratory survey is essential. This survey should include the examination of a selected series of examples from *both* the primary and the secondary xylem of lower vascular plants, gymnosperms, and angiosperms. Realistic study further requires the use of macerations in conjunction with the usual stained tran- and longisections of xylem. The following material is recommended for class study.

(*a*) *Tracheids.* Sections and macerations of the secondary xylem of conifers provide excellent illustrations of the tracheids of gymnosperms, with their prominent oval or circular *bordered* pits. For this study, the wood of *Pinus, Abies* or *Pseudotsuga* may be used. The tracheids of angiosperms should also be studied, utilizing such readily obtainable material as the secondary xylem of *Quercus, Platanus* and *Castanea.* To investigate the form and varied wall sculpturing of the tracheids of *primary xylem,* obtain longisections of the vascular bundles of young herbaceous stems and of leaves. Longisections cut with the aid of the CO_2-Freezing microtome and stained with phloroglucinol-hydrochloric acid provide realistic views of (1) the varied types of wall patterns and (2) the effects of elongation on the walls of the protoxylem elements. Prepared slides or sections of fresh material of the young stems of *Ricinus, Cucurbita, Trifolium*

and *Medicago* and of the petiole of celery are highly recommended as sources of material. It must be emphasized that in any study of the primary xylem of angiosperms, *both* tracheids and vessel elements (in varying proportions) may occur. Whenever there is doubt as to the existence of perforations, the cell in question may be designated descriptively as a "tracheary element."

(*b*) *Vessel elements.* The primary xylem of the concentric vascular strands in the rhizome of the bracken fern (*Pteridium latiusculum*) provides a classical example of vessel elements with primitive *scalariform perforations* (cf. Jeffrey, 1917, pp. 92-93, Fig. 71; and Bliss, 1939). *Obtain partially* macerated xylem of this plant and study the form, arrangement, and *scalariform bordered pitting* of the large tracheary elements. The sloping end-walls of these cells often show a series of narrow, transversely oriented, slit-like perforations. That these perforations represent pits, the membranes of which break-down during ontogeny, is clearly shown by *stained longisections* of the bundles. According to Bliss (1939, p. 620) in *Pteridium* "there are many cells that may be interpreted as transitional between the tracheid and the vessel element." *Scalariform perforation plates* are also typical of the vessel elements of numerous angiosperms. *Obtain* macerations of the secondary xylem of *Liriodendron, Betula* or *Alnus* and study the structure of the large conspicious scalariform perforation plates and the distribution and types of pitting on the lateral walls of the prominent vessel elements. Material for a comparative study of the more advanced *simple perforation* is furnished by a wide range of angiosperms. Among common woody plants, the secondary xylem of *Quercus, Vitis,* and *Malus* provide graphic illustrations. In the case of herbaceous plants the large vessel elements of *Cucurbita, Zea,* or *Apium* (cf. Esau, 1936 and Esau and Hewitt, 1940) may be used. The study of macerations should be accompanied by the examination of prepared stained sections of the xylem in each case. Such sections will illustrate the distribution of *vessels* and the fact that they are formed by a series of interconnected perforated elements. If time permits, a study should also be made of the vessel elements in macerations of the secondary xylem of *Ephedra.* In this genus, the sloping end-walls of the tracheid-like vessel elements

are provided with numerous small, oval, or circular perforations. As noted previously in this exercise (p. 119), these perforations evolved from the disappearance of the membranes of circular bordered pits.

2. *Origin and early development of primary xylem.* As an introduction to the more extensive study of the vascular system to be considered in Exercises XIII, XIV and XV, it will be desirable to study briefly at this point the origin and arrangement of the tracheary elements of the *protoxylem.* This tissue, as explained earlier, establishes the pattern, i.e., the direction of radial maturation, of the primary xylem as a whole. In the case of stems, *examine* stained tran- and longisections of early stages in the development of vascular bundles. Excellent material is provided by the early ontogeny of the vascular bundle in *Zea,* which is recommended for such a study by Esau (1943b). Transections of young vascular bundles in the stem of *Trifolium* should also be studied, since they illustrate the radial seriation of *both* proto- and metaxylem. Transections of early stages in the development of the *stele in roots* are very instructive since the tracheary elements of the protoxylem are clearly defined at the *outer edge* of each future primary xylem strand. In this case, as is also true very often in stems and leaves, the protoxylem elements differ from those of the metaxylem in their much smaller diameters.

3. *The cellular composition of paper.* A large proportion of paper is obtained from the wood of certain gymnosperms (e.g., *Abies, Picea*) and angiosperms (e.g., *Populus, Betula*). The first stage in the manufacture of paper from wood is the mechanical and chemical maceration of the xylem, which results in the partial dissociation of its component cells. This macerated xylem is commercially known as *wood pulp* and, after being bleached, colored, or "sized" according to the required use, is compressed under great pressure into paper sheets (cf. Sutermeister, 1941 for further details). It is interesting to note, however, that despite the drastic treatments involved in the production of wood pulp, many of the tracheary elements are well preserved and their structure and pitting are recognizable if small pieces of soaked paper are carefully teased apart in water and examined

under the microscope. Make a study of the various types of cells found in newspaper, blotting paper, and some cheap grade of writing paper.

IV. Suggested Drawings and Notes

1. Prepare drawings of individual tracheids, from the study of macerated gymnosperm and angiosperm woods, showing the form of the cells and the nature of the pitting.

2. Draw, on a large scale, a representative section of the entire primary xylem of *Medicago, Trifolium, Phaseolus, Cucurbita,* or *Apium* as seen *in longisectional view.* Show as carefully as possible the various types of secondary-wall patterns in the successive tracheary elements.

3. Prepare drawings of individual vessel elements in a series of plants to illustrate various types of perforation plates, variations in cell form and the character of the pitting on the lateral walls.

4. Illustrate by means of several drawings the position and structure of protoxylem tracheary elements, as seen in tran- and longisections of young vascular bundles (*Zea, Trifolium*).

5. Summarize, in tabular form, the various types of cells observed in the specimens of paper studied. Indicate whether differences in the kinds of tracheary elements observed can be used to deduce the source of the paper in each case, i.e., from conifer or angiosperm wood.

6. Prepare a brief summary of the relation of our present knowledge of vessel evolution and distribution to the problem of the origin and classification of angiosperms (cf. Bailey, 1944a and Tippo, 1946).

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Exercise XI

SIEVE CELLS AND SIEVE-TUBE ELEMENTS

The phloem of angiosperms, although very frequently complex in structure, is characterized by the consistent presence of a definite type of cell termed the *sieve-tube element*. This living cell is unique in that it is devoid of a nucleus at maturity. In the majority of angiosperms studied, the sieve-tube elements arise from the procambium and from the vascular cambium as more or less well-defined series of interconnected elements. To each of such series the term *sieve-tube* should be applied. Thus, from an *ontogenetic standpoint*, a sieve-tube closely resembles a vessel in being composed of a superposed series of closely related cells. The latter, however, in contrast to vessel elements, contain protoplasm, plastids, and a variety of "inclusions" during their functional life. The phloem of lower vascular plants and of gymnosperms likewise contains enucleate cells but these elongate, tracheid-shaped elements are not arranged in a well-defined superposed series. The term *sieve cells* may be used to designate these cells, which evidently are morphologically and functionally equivalent to the more highly specialized sieve-tube elements of the angiosperms.

In the phloem of angiosperms, the sieve-tube elements are usually accompanied, on *one or more of their lateral walls*, by much smaller cells known as *companion cells*. A companion cell differs from a differentiated sieve-tube element in the retention of its nucleus, in its denser cytoplasm, and by the absence of definite sieve plates. Companion cells are to be regarded ontogentically as sister cells of the sieve-tube elements since *both* types of cells originate by the longitudinal division of a common mother cell. A given companion cell may be equal in length to its associated sieve-tube element; frequently, however, the original companion cell may divide *transversely* one or more times, thus producing a series of companion cells next to the wall of the sieve-tube element (for further details on companion cells, cf. Esau, 1939, pp. 409-411 and Eames and MacDaniels, 1947, p.

106, Fig. 52). Companion cells, in the sense explained above, are not present in the phloem of gymnosperms; the so-called "albuminous cells" in this group of plants have been regarded as analogous with true companion cells. In addition to the definitive sieve-cells or sieve-tube elements and their companion cells, the phloem of some plants, especially the *secondary phloem* derived from the cambium, may contain in widely variable proportions other cell types such as parenchyma cells, sclereids, fibers, laticiferous tubes, and secretory elements. *Primary phloem* is often relatively simple in structure consisting of sieve-tube elements, companion cells, and phloem parenchyma, as in *Cucurbita*, or only the first two cell types may be present, as in *Zea* (cf. Esau, 1943b). Judging from a number of recent investigations, fibers may prove to be a very common cell type in the *protophloem* (i.e., earliest-formed portion of the primary phloem) in many angiosperms (cf. Essau, 1938; 1943c).

Following the discovery of the sieve-tube by Hartig (1837), attention rapidly began to center on the functional role of this structure. Today it is generally held that the living portions of phloem tissue are concerned with the transportation of organic solutes. Although experimental evidence, coupled with histological data, appears to support the belief that sieve-tubes represent the *main channels* for solute movement, the mechanism of such movement is a highly controversial issue and cannot be discussed in this book (Crafts, 1939a; 1939b; Schumacher, 1939). One important result of the lively interest in the function of the sieve-tube has been a renewed attack on the entire problem of phloem structure and development. Extensive critical reviews of the literature have been prepared by Esau (1939, 1943a); these reviews, together with Esau's own extensive investigations, form the basis for the following résumé of the salient features of structure and ontogeny of sieve-tube elements.

I. The Protoplast of Sieve-tube Elements

One of the most distinctive characters of the *mature* sieve-tube element is the absence of a nucleus. Numerous developmental studies have shown that, although the *young element* possesses a normal uninucleate protoplast, maturation is accompanied by

the eventual disintegration of the nucleus. Prior to its breakdown, the nucleus has been observed to increase significantly in size and to lose its chromaticity (Esau, 1939, p. 375 and 1947, p. 230, Figs. 21-27). Although enucleate, the cytoplasm of the sieve-tube element remains alive for some time, as evidenced by the continued deposition of callus on the sieve plates during late stages in ontogeny and at the approach of the functionless state.

Prior to the disintegration of the nucleus, the cytoplasm of the sieve-tube elements of many dicotyledons contains a variable number of discrete entities known as *slime bodies.* These structures, which vary widely in form and distinctness, gradually become dispersed and ultimately fuse into a mass of viscous material in the vacuole of the sieve-tube element. In elements that have been killed and fixed for study, this viscous, apparently proteinaceous material (together with the products formed by the disintegration of the nucleus) becomes coagulated, forming dense, funnel-shaped *slime plugs* near one or the other of the terminal sieve plates. Very recently Esau (1947) has described a highly interesting aspect of nuclear disintegration in the sieve-tube elements of *Rubus*, *Gossypium*, and *Eucalyptus*. In these plants, prior to the complete breakdown of the nucleus, one or more nucleoli are extruded into the cytoplasm. That these structures are not slime bodies, as was assumed for the case of *Rubus* by Engard (1944), is shown by the difference in their ontogeny and behavior; both kinds of structures occur side by side in the same element and are strikingly different in appearance (cf. Esau, 1947, Fig. 17 and Figs. 21-40). According to Esau, the extruded nucleoli in the plants studied remain "morphologically unchanged as long as the sieve-tube element exists as an intact cell." In addition to slime bodies and extruded nucleoli, sieve-tube cytoplasm very frequently contains colorless *plastids*, each of which contains carbohydrate granules. The latter give a wine-red color-test when stained with iodine and have been interpreted as starch grains (cf. Esau, 1939, pp. 384-386).

II. Sieve Plates and Sieve Areas

As a sieve-tube element progresses in its differentiation, certain portions of its primary wall become structurally modified.

Such wall areas, when observed in face view, exhibit a sieve or reticulate appearance and are termed *sieve plates*. During the earliest phases in the study of sieve tubes, it was believed that the spaces between the network of wall substance were open pores. But further investigations led to the idea that strands of living protoplasm, surrounded by collar-like depositions of *callus*, occupy the *pores* of a sieve plate. Whether the vacuolar material of contiguous sieve-tube elements is in direct communication through the connecting strands, however, is an open question, even after nearly a century of study on sieve-tubes (Esau, 1939, pp. 388-389). Further discussion of the highly controversial ideas of the nature of the connecting strands in sieve-plate pores and the physiological implications of their structural diversity are beyond the scope of this book. For detailed treatment the student is referred to the publications of Esau (1939) and Crafts (1932, 1939a, 1939b).

From a general morphological viewpoint, two types of sieve plates occur on the end-walls of sieve-tube elements in the angiosperms. In a variety of angiosperms, the sloping end-walls of the elements are provided with a series of individual sieve areas separated either by bars or by a network of wall substance. The term "compound sieve plates" is applied to this condition, which is found, for example, in the sieve-tube elements of *Nicotiana, Vitis, Eucalyptus* and *Cocos* (cf. Esau, 1938, 1947, 1948 and Cheadle and Whitford, 1941, p. 625, Figs. 1 and 4). Very commonly, however, in sieve-tube elements with transverse or slightly oblique end-walls, the latter are occupied by a large single or "simple sieve plate." A classical example of this condition is represented by the sieve plates in *Cucurbita* (cf. Crafts, 1939a p. 174, Fig. 1, 2, 4). According to the recent survey of Cheadle and Whitford (1941) and Cheadle (1948) the simple type of sieve plate has evolved phylogenetically from the compound condition in the metaphloem of monocotyledons.

Sieve plates or at least comparable structures also occur to varying degrees on the *lateral walls* between adjacent sieve-tube elements. Very frequently these plate-like areas appear more delicate in structure than the plates on the end-walls, and the terms "sieve fields" or "sieve areas" have been applied to them.

The exact nature of the wall connections between sieve-tube elements and companion cells and between sieve-tube elements and phloem parenchyma cells requires much further study. Esau (1938, pp. 386-387) found "one-sided sieve fields" between sieve-tubes and phloem-parenchyma cells in *Nicotiana*. She states: "In these the connecting strands and callus cylinders are very small, but may be detected in anilin-blue material. The heavy and chromatic part of the strand, as well as the callus cylinder, extends only through the sieve-tube wall." In the case of *Vitis*, Esau (1947, p. 227) found that the lateral wall of the companion cell next to the sieve-tube elements is provided with abundant pits.

From the standpoint of modern terminology (cf. Exercise II), the wall of the majority of sieve-tube elements is primary and consists of non-lignified cellulose. At the early *stages* in development of the sieve-tube element, prior to the disintegration of the nucleus, the wall is extremely thick (in sections of *fresh* undehydrated material) and has a more or less crenulated margin. French anatomists have adopted the term *"nacré"* to designate the wall at this stage of its development. As the sieve-tube element progressively matures and enlarges—processes attended by the loss of the nucleus and the degeneration of the slime bodies and plastids—the wall diminishes in thickness. Unable to resist the pressure of neighboring tissue growth, a *functionless* sieve-tube is often compressed or crushed or even, as in the case of the protophloem, entirely obliterated. In the secondary phloem of some plants, however, senile sieve-tubes, devoid of living contents, may remain open for a considerable period of time.

III. Definitive Callus

It has already been stated that the protoplasmic strands within the pores of sieve plates and sieve areas *early* become surrounded by *cylinders of callus*. As Esau (1939, pp. 387-388) has pointed out, the chemical nature of the callus of sieve plates is not yet clearly understood. Since the investigations of Wilhelm (1880), however, *anilin blue* has been widely employed in the study of sieve plates because this dye selectively stains the

callus but is not absorbed by the cellulose network of the plate. Continued differentiation in a sieve-tube element is usually accompanied by an increase in the amount of callus, the individual cylinders becoming laterally confluent. Finally each surface of the sieve plate becomes buried in a mass of callus, and the connecting strands become excessively stretched. At this stage in its development the callus is often designated as *definitive callus*. If the sieve-tube element is not crushed or obliterated at this time, the definitive callus eventually may dissolve completely away from the sieve plate. In some woody plants, the sieve-tube elements in the stem clearly function for more than one season. A striking example of this phenomenon is provided by *Vitis*. In this plant, according to the recent detailed investigations of Esau (1948), the sieve-tubes, which were formed during the preceding season, become "reactivated," i.e., the callus-pads disappear and the sieve-tube elements again become functional in the spring of the new season. Esau designates the callus that develops between two periods or seasons of activity in a sieve-tube as "dormancy callus," and reserves the term "definitive" to describe the callus present in the final stage when the sieve-tube becomes functionless.

IV. Primary Phloem and Secondary Phloem

As is true of the xylem (cf. Exercise X, pp. 123), no sharp boundaries can be drawn between the successively developed portions of the primary phloem nor between primary and secondary phloem. *Protophloem* marks the initiating point of subsequent differentiation of the primary phloem and originates during the early phases of organ elongation. *Metaphloem*, like metaxylem, is interpreted ontogenetically by Esau (1943a, p. 195) "as a tissue maturing largely toward the end of growth in length of the primary body." From a structural standpoint, protophloem may consist only of sieve-tubes that lack companion cells, as in the case of the vascular bundles of *Zea* (Esau, 1943b, pp. 339-340); in such examples, the sieve-tubes are crushed early and obliterated. In other plants, such as *Nicotiana* and *Linum*, cells adjacent to the protophloem sieve-tubes differentiate into fibers. No general distinction based on cell types can be drawn between

metaphloem and secondary phloem, although the former is often less complex histologically.

V. Material for the Study of Sieve-tube Elements

1. *The phloem of Cucurbita.* Because of the relatively large size of the sieve-tubes and their well-developed terminal sieve plates, the phloem of *Cucurbita* provides exceptionally good material for class study. A realistic study of phloem requires the use of fresh material and sections of the stem of *Cucurbita* can readily be cut with the aid of the CO_2-Freezing microtome. Crafts (1932) made a detailed study of phloem anatomy in *Cucurbita* and his paper will prove a valuable reference throughout this portion of the laboratory work.

The general anatomy of the stem should be studied first by treating a transection with phloroglucinol-hydrochloric acid and investigating it under low and high magnification. In progressing from the edge of the section towards the center the following tissues will be seen, viz.: (1) a typical uniseriate *epidermis*, certain cells of which have developed hairs; (2) a rather narrow *cortex*, consisting of an outer discontinuous zone of "angular" *collenchyma* followed by a zone of *parenchyma*, the innermost layer of which may contain abundant starch grains and appear as a *starch sheath* if the section is stained with IKI solution; (3) the *vascular system*, the outer boundary of which is clearly indicated by a continuous cylinder of thick-walled *fibers*. Internal to the fibers occurs a ground tissue of parenchyma embedded within which are usually ten prominent vascular bundles, arranged in two series of five bundles each. The center of the stem is occupied by a prominent cavity produced by the collapse and disintegration of the pith. Confirming the earlier studies of Fischer, Crafts (1932, p. 186, Fig. 1) depicts a series of "ectocyclic" and "entocyclic" sieve-tubes situated respectively near the outer and inner edges of the cylinder of fibers; furthermore "commissural" sieve-tubes, connecting the entocyclic tubes with the phloem of the outer vascular bundles and the peripheral tubes of the latter with one another, are also present. Note that each vascular bundle consists of a median strand of xylem (well marked out by the large vessels) flanked externally and internally

by a strand of phloem tissue. A bundle of this type is designated as a *bicollateral bundle*. Especially in the case of the larger bundles, a well-defined strip of *cambium* occurs between the xylem and each of the phloem strands. *To investigate the structure of the phloem*, obtain a fresh transection of the stem and mount it in a .1% aqueous solution of anilin blue.[1] This dye will stain the callus depositions on any of the sieve plates that may be present in the section. A careful study, *under high magnification*, of the phloem of the various bicollateral bundles will usually reveal a number of large *sieve plates*. These structures in *Cucurbita* occupy virtually the entire end-wall of the cylindrical sieve-tube elements. In critically stained sieve plates, each "pore" is occupied by a dark central spot, representing the large, connecting strand, and is surrounded by a distinct *callus-cylinder* stained a light blue. If *definitive-callus* has not yet appeared, the portions of the plate between the callus-cylinders should appear as an unstained network of wall material. Often, however, a sharp demarcation of this sort is not seen because of a more or less uniform coating of callus over the entire surface of the sieve plate. Note that in addition to the sieve-tubes, smaller *companion cells* are present and are distinguished by their nucleated protoplasts and their triangular or quadrangular form as seen in transection. *Phloem parenchyma* is also present but is difficult to distinguish from sieve-tube elements unless the latter exhibit sieve plates. This study of phloem should be continued with the aid of *longisections*, which likewise should be stained in anilin blue. Note carefully the appearance and structure of the sieve plates as seen in sectional view, and the relation of the sieve-tube elements to the companion cells and the phloem parenchyma. Often *slime-plugs* will appear in certain of the sieve-tube elements. They can readily be induced by treating the section with 70% alcohol.

2. *The phloem of Nicotiana or Vitis.* Obtain freshly cut longisections and stain them with anilin blue, noting the prominent *compound sieve plates* on the inclined end-walls of the sieve-tube elements. Tran- and longisections of the secondary phloem of *Vitis* provide excellent material (1) for the study of "*dormancy*

[1] Cf. Appendix p. 217-218.

callus" and (2) for a study of the phenomenon of reactivation in sieve-tubes (cf. Esau, 1948).

3. Obtain prepared transections of young stems of *Zea* and study the position and ultimate obliteration of the protophloem sieve-tubes in the developing vascular bundles. The *metaphloem* of each bundle consists of persistent sieve-tubes accompanied by small, well-developed companion cells.

4. Observe demonstrations of prepared longisections of the secondary phloem of a gymnosperm (e.g., *Pinus*), noting the long *sieve cells* with their numerous delicate *sieve areas*.

VI. Suggested Drawings and Notes

1. Diagram the general structure of a bicollateral bundle from the stem of *Cucurbita* as seen in transection. Label all essential parts and indicate by circles the position of the largest vessels of the xylem.

2. Draw small portions of the phloem tissue of *Cucurbita* as seen in both trans- and longisectional views. Show carefully the structure of at least one sieve plate in each drawing. Label all cell types and structures.

3. Prepare drawings of small portions of the phloem of *Nicotiana* or *Vitis*, to show the form and structure of the sieve-tube elements and their associated companion cells.

4. Outline, on a large scale, the contour of a young vascular bundle of *Zea*, as seen in transection, filling in the cellular details of the protophloem and metaphloem.

5. Summarize, with the aid of cell diagrams, the successive phases of differentiation of a sieve-tube element, from its initiation until the development of definitive-callus (cf. Esau, 1939, pp. 402-403).

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Exercise XII

LATICIFEROUS TUBES

One of the distinctive anatomical characteristics of many angiospermous genera is the presence in certain regions of the plant body of a complex series of tubes that contain a viscous fluid known as *latex*. De Bary's (1884, p. 183) term *laticiferous tubes* appropriately designates these structures. When plants that develop latex are cut, this fluid exudes more or less freely and, depending upon the species, appears milky white, yellowish, or colorless. Common illustrations of plants that contain latex are the "rubber plant" (*Ficus elastica*), "milk weed" (*Asclepias* sp.), dandelion (*Taraxacum*), lettuce (*Lactuca* sp.), and poinsettia (*Euphorbia pulcherrima*). The latex of certain species of plants is of great economic importance. Thus, for example, commercial rubber is derived from the latex of *Hevea braziliensis* and *Taraxacum kok saghyz*, whereas the latex of the poppy (*Papaver somniferum*) and the sapodilla tree (*Achras Zapota*) respectively yield opium and chicle.

From a chemical standpoint, latex is a complex suspension (or in some cases an emulsion) which varies widely in its composition. The suspended materials include rubber particles, waxes, resin, proteins and, in certain species of *Euphorbia*, starch grains. (For detailed reviews on the chemical nature of various latices, cf. Moyer, 1937, and Bonner and Galston, 1947.) The role of latex in the life of the plant is obscure, however, despite the numerous investigations that have been devoted to the solution of this problem (cf. Moyer, 1937, pp. 528-532). Haberlandt (1914, pp. 336-345) maintains that in many plants the latex is a "nutritive fluid" and for this reason he classifies laticiferous tubes under the "Conducting System." In contrast, Sperlich (1939), although recognizing the possible nutritional value of certain of the components of latex, assigns a storage function to laticiferous tubes. It seems evident from Sperlich's review of the literature that further insight must await the results of careful experimenta-

tion. One of the difficulties that has obstructed progress along experimental lines is the intimate association in many plants (e.g., *Hevea braziliensis*, *Taraxacum*) of the laticiferous tubes with phloem. Obviously the conventional type of "ringing" experiments widely employed in the study of translocation are difficult to interpret in such cases, especially because of the occurrence of laticiferous tubes in the *internal phloem* and the pith of many plants (cf. Moyer 1937, pp. 526-528).

I. Morphology and Ontogeny of Laticiferous Tubes

On the basis of differences in development and adult form, De Bary (1884, p. 186) recognized two principal categories of laticiferous tubes, namely, the *articulated* and *non-articulated*. Subsequent comparative researches justify the convenience of this classification although, as will be pointed out below, these types do not necessarily coincide with systematic relationships in plants. Since detailed reviews of the voluminous literature on the morphology of laticiferous tubes are given by De Bary (1884), Schaffstein (1932), and Sperlich (1939), the following discussion is presented simply as a guide to some of the salient facts that have been established.

1. *Non-articulated laticiferous tubes.* These structures are characteristic of members of the Apocynaceae, Asclepiadaceae, Euphorbiaceae, and Moraceae, and are among the most remarkable of all the cellular structures found in angiosperms. Much uncertainty prevailed in the past regarding the origin of these structures because in the fully developed plant the tubes very commonly form a complex, ramified system extending through various tissues in both the shoot and the root. Confirming the general account given by De Bary (1884), Schaffstein (1932, pp. 200-201, Figs. 1-2) has found that in certain species of *Euphorbia* the entire system of branching tubes originates from a relatively few initial cells in the embryo. These initials arise at the cotyledonary node at the outer edge of the meristematic vascular cylinder and send tubular processes upward into the cotyledons and downward into the hypocotyl and radicle. The subsequent path of development of the tubes following their early development in the embryo varies according to the particular

species of plant. In certain species of *Euphorbia*, for example, the main laticiferous tubes lie at the periphery of the vascular cylinder and send branches outward into the developing leaves and buds and inward into the pith. In contrast, the primary latex tubes of *Ceropegia Twaitesii* are medullary, and branching from them into the cortex and leaf primordia occurs at the nodes (cf. Schaffstein, 1932, p. 205, Fig. 5). According to the investigations of Blaser (1945) on *Cryptostegia grandiflora* the primary cortical tubes branch radially at the level of the prominent leaf gaps and to a lesser degree within the embryonic vascular rays. As a result, latex tubes extend into the pith and hence become "embedded" within the secondary phloem and xylem following cambial activity. Aside from these variations, the essential fact is that the extension and branching of non-articulated laticiferous tubes occur *continuously* during the entire development of the plant, so that new leaves, buds, and lateral roots are progressively "invaded" by the growing ends of the tubes. Although exact measurements are lacking, the total length of the tubes in a large plant, for example *Euphorbia*, must be enormous. According to Schaffstein (1932), although the greatest increase in length of the tubes occurs through "secondary stretching" in correlation with the elongation of neighboring tissue elements, *the tips of the tubes* exhibit true apical growth. This is normally restricted, in the shoot axis, to a region a short distance behind the apical meristem. In such meristematic tissue, the terminal growing tips of the tubes end blindly. It is of interest to note that the older portions of the walls of non-articulated tubes in certain plants retain a marked capacity for growth and the development of lateral branches. Good evidence for this is provided by *grafting experiments* with *Euphorbia esculenta*. Schaffstein (1932, p. 215, Fig. 10) found that new branches are sent out from the cortical tubes of the scion into the meristematic region of the "secondary callus" at the point of the graft union.

In concluding this brief summary, mention must be made of the multinucleate character of the non-articulated type of laticiferous tube. Treub (1880) recorded a plurality of nuclei in the latex tubes of a number of genera, and Schaffstein (1932) observed that this condition arises during the earliest phases of

ontogeny in several species of *Euphorbia*. In these cases at least, it seems clear that each profusely branched latex tube is a *coenocyte*. But whether nuclear divisions continue in the distal portions of the tubes or whether some of the nuclei fuse and degenerate, as in certain bast fibers (Esau, 1938), represent problems yet to be solved. According to Moyer, (1937, p. 522) nuclei have been found in the exuding latex by a number of investigators.

2. *Articulated laticiferous tubes.* These structures are distinguished ontogenetically from the preceding category by the fact that they arise from the partial or complete dissolution of the transverse walls of a continuous series of cells. Thus an articulated tube is, like a vessel, a compound structure resulting from the coalescence of many cells. In some plants (e.g., *Allium* and *Achras Zapota*), a series of laterally unconnected laticiferous tubes are developed (Karling, 1929). Very commonly, however, the tubes are early connected by anastomoses and hence at maturity appear as a more or less complex reticulum (cf. De Bary, 1884, p. 190, Figs. 82-83; Sperlich, 1939, p. 153, Fig. 43).

As in the case of the non-articulated tube, the articulated type originates early in the ontogeny of the plant. Fusion of cells has been observed in the embryos of *Tragopogon* and *Scorzonera* by Scott (1882) and in the embryo of *Hevea braziliensis* by Schaffstein (1932). In the Russian Dandelion (*Taraxacum kok saghyz*), Artschwager (1943) found that the tubes, which develop within the pericycle and primary phloem of the root, "make their appearance soon after seed germination." Although further investigations on a wide range of plants are highly desirable, the cells that coalesce to form articulated laticiferous tubes very commonly are the undifferentiated elements of the "pericycle" and phloem. In addition to the dissolution of the end-walls in a superposed series of cells, portions of the side-walls between two laterally adjacent tubes may also disappear, resulting in the interconnection of originally discrete tubes. The more conspicuous transverse anastomoses are developed in two ways, viz.: (1) by the coalescence of the members of a transverse or oblique row of cells that intervene between two vertically oriented tubes and (2) by the development of re-

markable lateral branches that protrude from the lateral walls of the main tubes. These lateral protuberances, which were compared by Scott (1882) to the conjugation tubes of algae, may meet and fuse in pairs within the tissue separating two vertical tubes, thus producing a cross-connection. Some of the lateral branches, however, may fail to pair and instead may end blindly in the tissue.

As mentioned earlier in this résumé, the distribution of the two types of laticiferous tubes does not entirely coincide with systematic relationships. The best example of this is furnished by the situation in the Euphorbiaceae. In this family, the majority of the genera investigated develop the non-articulated type of tube. But in the economically important *Hevea braziliensis* and *Manihot Glaziovi,* which belong to the same family, the tubes are of the articulated type. In addition to these species, members of the following families are reported as possessing articulated laticiferous tubes: Olacaceae, Papapaveraceae, Caricaceae, Campanulaceae, Lobeliaceae, and the tribe Cichorieae of the Compositae.

A very interesting type of *unbranched* non-articulated latex tube occurs in *Cannabis* (Zander, 1928) and in *Vinca minor* and *Urtica dioica* (Schaffstein, 1932). According to Schaffstein's ontogenetic investigation, the embryo of *Vinca* is devoid of laticiferous tubes. On the contrary, in the shoot each tube originates from a solitary initial situated at the outer edge of the procambium below the point of insertion of a leaf primordium; from the region of origin, the tip of the tube advances upward into the leaf, its growth ceasing when the leaf reaches maturity (Schaffstein, 1932, p. 210, Fig. 8).

II. Material and Procedures for the Study of Laticiferous Tubes

1. Make incisions into leaves and stems of assembled plant material (*Ficus elastica, Euphorbia pulcherrima, Euphorbia splendens, Nerium oleander, Sonchus oleracea,* and *Lactuca scariola*) and observe the flow of latex. Collect some of the latter on a slide, add a cover slip, and study the microscopic nature of the latex. The *Ficus* latex is composed of coarse

granules; that of *Euphorbia pulcherrima* and *E. splendens* is very finely granular but contains large starch grains (spindle-shaped, rod-shaped, rod-shaped with swollen ends; rarely roundish in outline); in *Ficus, Euphorbia* and others the latex has the appearance of dense, white milk; in oleander, however, it is only slightly cloudy. Some plants have yellow or brown latex (*Cannabis*, Hayward, 1938, p. 239). The degree of cloudiness of latex varies according to the species and age of the plant part. *Prepare drawings* illustrating the granular latex of *Ficus* and the starch grains of *Euphorbia.*

2. *Non-articulated laticiferous tubes.* The following materials are recommended for laboratory study:

(*a*) *Sambucus:* unbranched non-articulated tubes. The contents of these tubes become dark brown with age and consist of resins, gums, and tannins. Thus the contents differ from typical latex but appear to resemble the contents of the latex tubes of *Cannabis.* Dissect out from partly macerated stem-pieces the tubes that occur in the phloem and pith and have dark brown contents. The tubes are not interconnected. They arise from single cells, become very long (2 cm. or more, De Bary, 1884, p. 148), and have tapering ends. Prepared slides of trans- and longisections of *Sambucus* stem should be used in connection with the study of macerated material.

(*b*) *Euphorbia splendens:* branched non-articulated latex tubes. Dissect out the latex tubes from the cortex of the partly macerated (by boiling a few minutes in weak NaOH) stem of *Euphorbia splendens.* Place portions of the tubes on a slide and study the microscopic details. *Prepare a drawing* showing the method of branching of the tubes.

(*c*) Cleared leaf-pieces of *Croton.* Prepared slide. This slide shows an unsectioned and unstained piece of cleared leaf in which the thin-walled elements have become almost transparent. The elements that did not become very clear are (1) the xylem elements, which traverse the leaf as anastomosing bundles, and (2) the fiber-like sclereids. The laticiferous tubes (branched non-articulated tubes) are partially cleared and, when the light is dimmed by means of the condenser diaphragm, the tubes may be recognized as thin-walled branching structures containing

some yellowish-granular coagulated material—the latex. The main branches of the tubes are wider than the sclereids, the smaller branches are narrower. Follow some of these tubes to their ends and observe the blind endings.

(*d*) *Latex cells of Allium* (De Bary, 1884, p. 147). Some groups of plants have elements that are closely related to the latex tubes, though they show no elaborate branching or anastomosing, nor are they extraordinarily long. In the latter instance the individual cells are interconnected by pits. Whether the walls become perforated is not definitely known. A good example of such latex cells may be found in the onion. The chains of latex cells occur between the second and third layers of parenchyma beneath the abaxial epidermis of leaves and scales. Examine a portion of an onion scale boiled in water. The opaque cells that are arranged in parallel longitudinal rows are the latex cells. They are filled with cloudy granular fluid. Remove the epidermis and attempt to force the contents of the latex cells from one to the other. The end-walls do not appear to permit the passage of the granular material. *Draw* a row of latex elements and adjacent parenchyma cells to show the relative length of the two kinds of cells and the manner of connection among the latex cells. *Prepare* a transverse section of an onion scale, stain it in neutral red, and locate the latex cells. *Draw* an end-wall of a latex cell seen in face view and show the nature of the pitting.

3. *Articulated laticiferous tubes.* A good illustration of articulated laticiferous tubes is found in *Lactuca scariola*, the prickly lettuce. Study as follows:

(*a*) Secure a prepared slide of the transections of stem of *Lactuca scariola.* The general structure of the stem is as follows: The outermost cell layer constitutes the epidermis. Then follows the cortex, which is collenchymatous in its outer region. The vascular bundles are discrete and occur in a circle around the pith. There is external phloem (outside of the xylem) and internal phloem (inside of the xylem). The laticiferous tubes occur on the outer border of the outer phloem and within the phloem itself (external and internal). The largest tubes are those present on the outside of the phloem. Locate these elements. They occur about 1-3 cell rows inside of the "starch sheath," a

layer of closely connected cells containing much starch. The laticiferous tubes have relatively thick walls and somewhat shrunken contents. Some tubes are interconnected. Draw a diagram showing the general structure of the stem and indicating the position of the laticiferous tubes. *Draw* several tubes and adjacent cells at high power.

(*b*) Obtain a partly macerated (by boiling a few minutes in weak NaOH) stem of *Lactuca scariola*. By the use of a dissecting microscope dissect out the laticiferous tubes located on the outer border of the phloem. Because of interconnections, the tubes form a dense continuous network. Make a low-power diagram illustrating this net. Place a portion of the net on a slide, add a cover slip, and study at high power. *Draw* to show the nature of interconnections between the tubes.

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Exercise XIII

THE STEM

In this and the two following exercises, a brief study will be made of the comparative anatomy of the three principal vegetative "organs" of the sporophyte of seed plants, viz.: the stem, leaf, and root. Paleobotanical evidence shows clearly, however, that this conventional subdivision of the plant body cannot be applied to the Psilophytales, generally regarded as the most primitive of all tracheophytes. Indeed, in the Psilotales, which are the living representatives of this ancient group, roots are absent and the aerial portion of the sporophyte is not clearly demarcated into stem and leaves. Furthermore, it is clear that even in seed plants the boundary between stem and leaf can only be made rather arbitrarily. Both of these "organs" arise from a common terminal meristem (i.e., the shoot apex) and their further differentiation and growth is reciprocal and interdependent to a large extent (Esau, 1943b, p. 254 and Wetmore 1943, p. 25). For these reasons, it seems preferable, from a morphological standpoint, to include both the axis (i.e., the stem) and its foliar appendages under the broader concept of the *shoot*. This concept has found application not only to the vegetative region but has also been widely adopted in the anatomical interpretation of the flower of angiosperms (cf. Eames and MacDaniels, 1947, pp. 341-360). Hence, in this book, the separate treatment given to the stem and the leaf is largely a matter of practical convenience and its limitations on morphological grounds should be constantly borne in mind (cf. Arber, 1941, for a penetrating discussion of the problem). The *root,* which is axis-like in form, clearly deserves separate discussion and study and its anatomical features will be outlined briefly in Exercise XV.

I. The Primary Structure of the Stem

In the majority of the lower vascular plants and in certain herbaceous angiosperms, all the stem tissues are "primary,"

i.e., they originate from the protoderm, ground meristem, and the procambium. In accordance with this fact, a common plan of *primary structural organization* is found in the "typical" stems of vascular plants. The following tissues and tissue-zones are recognized:

1. *The epidermis.* Stems possess a well-defined epidermis in which stomata, idioblasts, and various types of trichomes may be present in addition to the typical epidermal cells (for a detailed description of the histology of the epidermis, cf. Exercise V). In herbaceous plants, the epidermis may persist throughout the life of the stem; in woody plants, however, it is ultimately destroyed and sloughs off as a result of the development of periderm.

2. *The cortex.* Beneath the epidermis of stems is found a cylindrical zone of tissue, variable in its radial dimension and in the types of component cells. This region is the cortex and, in the simplest condition, consists entirely of thin-walled *parenchyma tissue;* in many stems, such cortical parenchyma may function in both photosynthesis and in the temporary storage of starch and other metabolic products. Often, however, the cortex is histologically more complex and exhibits an outer subepidermal zone of *collenchyma* or *fibers* (either of which may develop as a continuous cylinder of cells or as discrete strands) and an inner region of parenchyma. Other cells types may also be present, particularly various types of *idioblasts* (e.g., sclereids, secretory cells) and, in certain angiosperm families, laticiferous tubes (cf. Exercise XII). From the standpoint of Sachs' tissue systems, the cortex represents the outer part of the "fundamental tissue system."

3. *The primary vascular system.* Internal to the cortex and in some plants in direct contact with it, there occurs the primary vascular system of the stem. In gymnosperms and the majority of dicotyledons, the vascular system is either a continuous or a dissected *cylinder* of phloem and xylem that encloses a central *pith* region. Most commonly, this cylinder consists of an external layer of phloem and an internal layer of xylem. In the case of a dissected cylinder of this kind, each vascular bundle is of the *collateral type.* But in certain angiosperm families (e.g.,

Solanaceae and Cucurbitaceae) *internal phloem* occurs as well, either in the form of discrete strands at the outer edge of the pith or in direct contact with the inner edge of the primary xylem. The stems of certain angiosperms, e.g., many monocotyledons, do not exhibit, however, a definable "vascular cylinder," the primary vascular system consisting of a large number of irregularly arranged vascular bundles. In such stems, no boundary can be drawn between the cortex and the pith regions.

Aside from its great physiological interest, the primary vascular system, because of the variations in its structural patterns in the main groups of vascular plants, has been widely investigated comparatively in both living and extinct plants. In an effort to provide a unified morphological interpretation of the "plant axis" (i.e., the stem and the root) Van Tieghem and Douliot (1886) proposed and developed the *stelar theory*, which has profoundly influenced all subsequent investigations in comparative anatomy. Stated very briefly, this theory postulated that root and stem are fundamentally alike in gross anatomy, since in each the cortex encloses a central core or *stele*. The latter consists of the pericycle, the vascular system, and the pith (when present). Thus, for example, in the stem of a dicotyledon the term *stele* would designate *collectively* all the primary tissues lying internal to the cortex. Although the subsequent development of the stelar theory cannot be discussed in any detail in this book, certain matters of general terminology and concept merit brief attention at this point (for extended treatments of the stelar theory, cf. Jeffrey, 1903; Brebner, 1902; Eames and MacDaniels, 1925, pp. 337-340; and Bower, 1935). It is now rather generally held that two *principal* types of stele occur in the sporophyte of vascular plants, viz.: (1) the *protostele*, which consists of a central core of xylem (devoid of pith) ensheathed by phloem (the protostele, occurring in the Devonian Psilophytales and many of the living lower vascular plants, is believed to represent the most primitive condition); and (2) the *siphonostele*, which is characterized by the presence, internal to the primary xylem, of a central column of pith. The siphonostele is regarded as derived phylogenetically from the protostele and is the prevailing type of

stele in the living members of the Pteropsida (i.e., the ferns, gymnosperms, and the angiosperms). As seen in transectional view, the vascular tissues of a siphonostele appear either (1) as a continuous "ring" of xylem ensheathed by the phloem or (2) as a "ring" of more or less widely separated vascular bundles. A siphonostele of the latter type should be visualized as a *tubular network* of interconnected bundles separated from one another by vertical strips of parenchymatous tissues (cf. Esau, 1943b and 1945). The term *dictyostele* has been widely applied to the "dissected" type of siphonostele (Eames and MacDaniels, 1947, p. 141). But in a recent critical discussion of stelar nomenclature, Nast (1944, p. 455) points out that the term *dictyostele* was originally used by Brebner (1902) for a special type of stele occurring in the ferns. To designate the dissected type of stele that occurs so commonly in the angiosperms, Nast recommends Brebner's term *eustele*. In gymnosperms and dicotyledons, the siphonostele is specifically termed *ectophloic*, if there is only an external area of phloem, or *amphiphloic*, if both internal as well as external phloem occur.

The stelar theory has proved extremely helpful in the description and interpretation of stem anatomy, particularly in the lower vascular plants. But certain modern investigations have indicated the urgent need for a complete re-examination of the concept of stele, especially in the case of seed plants. It may be properly asked: What constitutes the *outer boundary* of the stele in the axis of a dicotyledon? The answer to this question seems to depend upon the occurrence and the nature of two "tissues," viz.: (1) the *endodermis* and (2) the *pericycle*. In the majority of roots and in the stems of some vascular plants, the innermost parenchymatous cells of the cortex abut directly upon a uniseriate layer of cells known as the endodermis. This is composed of living cells, the radial and transverse walls of which are provided with bands of "suberized" material known as *Casparian Strips* (for further discussions of the endodermis, cf. Exercise XV). Van Tieghem apparently considered the endodermis as the *innermost layer of the cortex*, although certain modern authors (e.g., Eames and MacDaniels, 1947, p. 161) consider it as "the limiting layer of the stele." Ontogenetic studies,

however, have not been decisive in favor of either of these viewpoints. For this reason, and because of its sporadic occurrence in the stems of seed plants, the endodermis obviously cannot be *consistently* used to set the boundary between the cortex and the stele. Enclosed by the endodermis (or by the innermost layer of cortical cells) occurs the so-called *pericycle,* which most authors interpret as the outermost portion of the stele. In roots, the pericycle is usually an uniseriate layer of active cells found in direct contact with the protophloem and protoxylem; pericycle cells in roots participate in the formation of the cork and vascular cambium and give rise to the primordia of lateral roots. But in the *stems* of angiosperms, it has proved impossible, in a number of species, to demonstrate a "pericycle" at the outer edge of the primary vascular system. In *Nicotiana, Linum,* and *Helianthus* for example, the so-called *pericyclic fibers* prove to originate and to develop within the protophloem; in these forms, therefore, the outermost edge of the primary phloem is in direct contact with the cortex and no definable pericyclic region occurs (Esau, 1938, 1943d, 1945). The investigations of Esau and her thorough review of the literature (1943a, 1943d) make it plain that the entire concept of "pericycle," for *both* roots and stems, demands reinterpretation on an ontogenetic basis.

In the light of the above critique it should be evident that the nature of the morphological boundary between cortex and stele presents a series of problems awaiting ontogenetic study. For this reason and because of the highly controversial state of stelar nomenclature (cf. Nast, 1944, pp. 454-456), the expressions "primary vascular system" or "vascular cylinder" seem preferable to "stele" and will be employed in this exercise.

4. *The pith.* The center of the stem is occupied by a column of tissue termed the *pith.* Histologically, the pith varies widely as to the types of its component cells. Often it consists only of parenchyma, but sclenchymatous and secretory idioblasts, laticiferous tubes and "medullary" vascular bundles may be present. Ontogenetically, the pith develops from the ground meristem and may be regarded as the inner portion of the "fundamental tissue system." In many herbaceous plants, the rapid elongation and radial expansion of the stem result in the partial or complete

destruction of the pith early in ontogeny (cf. Esau, 1943b, p. 742, Figs. 6-12).

II. The Nodal Anatomy of the Stem

The continuity of the vascular cylinder is interrupted at each node by the divergence of vascular strands or "traces" to the leaf and its axillary bud or buds. The "departure" of leaf traces is associated, especially in stems that have already developed some secondary vascular tissues, with well-defined parenchymatous areas designated as *leaf gaps*. The comprehensive survey of Sinnott (1914) revealed that three distinct types of nodal anatomy (with respect to the leaf) occur in the stems of dicotyledons, viz.: (1) the *unilacunar type*, in which the vascular trace or traces of *each* leaf produce a single gap; (2) the *trilacunar type*, characterized by the formation of a "median" and two "lateral" gaps; and (3) the *multilacunar type*, in which more than three gaps are produced. These types of nodal anatomy have proved of considerable value in studies of the phylogeny of dicotyledons, since a given type is often consistent through a genus or even a family (Sinnott, 1914; Sinnott and Bailey, 1915; Bailey and Howard, 1941a; Bailey and Nast, 1944). Extensive investigations by Bailey support the idea that the trilacunar condition is primitive for dicotyledons; from it, respectively by reduction and amplification, have arisen the more advanced unilacunar and multilacunar nodes. Wide application of the principles of nodal anatomy has also been made in the study of the vascular anatomy of the flower (cf. Eames, 1931, and Eames and MacDaniels, 1947, pp. 342-356).

According to Eames and MacDaniels (1947, p. 144), the primary vascular supply to a branch usually consists of one or of two *branch traces*, which are associated with a single *branch gap*. In many trilacunar dicotyledons, the two branch traces appear to be attached at either side of the gap produced by the median leaf trace. Very few complete studies, however, have been made on the vascularization of axillary buds and, as Miller and Wetmore (1946, p. 8) point out, "a wide variety of structural types must be investigated before any generalizations are possible."

III. The Origin and Development of the Primary Vascular System

Within recent years there has been a marked revival of interest in the origin and differentiation of the primary vascular system of the stem. Esau (1943a) has critically reviewed the extensive literature and through her own investigations contributed significantly to this important field of research. Within the limited scope of this book it will only be possible to summarize the salient features of the subject. One of the most important results of recent investigations has been the re-emphasis on the interrelationship between leaf initiation at the shoot apex and the continuous *acropetal development* (i.e., upward differentiation) of procambial strands in the terminal portion of the shoot. In other words, apical growth in the shoot is accompanied by the progressive development of new procambial strands, each of which is distally connected with an older strand and, in its further upward growth, is destined to become a leaf trace. According to several very recent investigations, the acropetal development of a new procambial strand may be recognizable before the leaf primordium, into which it ultimately will differentiate, has emerged as a definable protuberance on the shoot apex. This precocious development of procambium has been reported in a number of gymnosperms (cf. Crafts, 1943a, 1943b; Sterling, 1945, 1947; Gunkel and Wetmore, 1946a, 1946b) and is admitted as a distinct possibility for *Linum* by Esau (1943b, p. 254). Sass (1944, p. 455), who found this condition in the shoot tip of tulip, properly raises the question "whether the inception of a strand is the precursor or the consequence of leaf initiation." This difficult matter may ultimately be solved through experimental work with the shoot apex (cf. Snow and Snow, 1947).

Recent investigations are in rather general agreement with regard to the pattern of *longitudinal development* of vascular tissues from the procambium in the apical region of the shoot. In gymnosperms, as well as in a number of dicotyledons, the direction of development of the *protophloem* is, like that of the procambium, strictly acropetal; well-defined protophloem elements (in continuity with more distal phloem) can be detected at the outer edge of a given procambial strand soon after the emergence

of its associated leaf primordium. In marked contrast, the *protoxylem* arises later than the protophloem and at first consists of a *discontinuous group of tracheary elements* situated at the inner edge of a procambial strand in the basal region of the young leaf. From this point, the further longitudinal maturation of the protoxylem occurs both *basipetally* (i.e., downwardly) until union is made with the xylem of an older strand, and *acropetally* toward the leaf apex. In order to visualize the contrast between the continuous acropetal development of the procambium and protophloem, and the characteristic discontinuity of the first xylem, the student should study the helpful diagrams found in the papers of Esau (1938, p. 354, Fig. 4; 1943b, p. 251, Fig. 9; 1945, p. 25, Fig. 24), Crafts (1943a, p. 118, Fig. 36), and Miller and Wetmore (1946, p. 2, Fig. 3).

With reference to the *progressive radial maturation* of the primary phloem and primary xylem within a procambial strand, the primary phloem differentiates *centripetally* (i.e., toward the center of the stem) whereas the primary xylem differentiates *centrifugally*. Primary xylem developing in this manner is termed *endarch* and is characteristic of the stem of the seed plants.

IV. The Secondary Structure of the Stem

As stated previously, the stems of many vascular plants consist exclusively of tissues produced by the protoderm, ground meristem, and the procambium. But in gynosperms and woody dicotyledons, there are superimposed upon the so-called "primary body" of the stem additional tissues derived from the *vascular cambium* and from the *phellogen* or cork cambium. Tissues derived from such cambia are commonly designated as "secondary tissues"; as pointed out in Exercise X, however, the *morphological distinction* between "primary" and "secondary" vascular tissues is somewhat arbitrary and depends upon the criteria used for distinguishing the procambium from the vascular cambium. Sustained cambial activity in stems eventually results in the complete elimination of all primary tissue zones with the exception of the primary xylem and the pith. These portions of the "primary body" become completely enclosed by the cylinder of secondary xylem unless already destroyed by stretching and compression

during stem elongation. The processes of secondary growth in stems are extremely complex and the following brief discussion is intended merely as an introductory guide. (For a detailed treatment, cf. Eames and MacDaniels, 1947, Chapters VI, IX and XI.)

1. *The vascular cambium.* In a strict sense, the vascular cambium should be regarded as a uniseriate layer of meristem composed typically of *ray initials,* which produce the *vascular rays,* and *fusiform initials,* which give rise to the vertically oriented cells of the secondary phloem and secondary xylem. In examining the cambium of actively growing stems in transectional view, however, it proves difficult or impossible to distinguish the true "cambial initials" from their recent phloem and xylem derivatives. The latter at first possess only primary walls like the cambium and moreover have the capacity for further tangential division. For these reasons, the cambial initials and their adjacent immature phloem and xylem derivatives may be collectively designated as the "cambial zone."

(*a*) *Fascicular and interfascicular cambium in gymnosperms and dicotyledons.* Regardless of the ultimate pattern of the secondary vascular cylinder, the first formed phloem and xylem in the stems of seed plants originate respectively at the outer and inner edges of discrete procambial strands. From these "poles," as noted previously, the further maturation of primary phloem and primary xylem proceeds radially toward the middle of the young vascular strand. In monocotyledons and certain herbaceous angiosperms, the metaphloem and metaxylem ultimately meet, and the growth of the vascular bundle ends (Esau, 1943c). But in gymnosperms and the majority of angiosperms, the procambial cells in the young bundle between the strips of metaphloem and metaxylem continue to grow and divide; from them are derived, often by a gradual transition, the cells of the vascular cambium (cf. Sterling, 1947). For convenience, the strips of cambial tissue arising in this way are designated collectively as the *fascicular cambium.* In certain dicotyledonous herbs, cambial activity is restricted to the original vascular bundles, and the interfascicular areas of the vascular cylinder develop into vertical strips of parenchyma. On the other hand, in many types of

woody and herbaceous stems, strips of *interfascicular cambium* develop *between* the original bundles and become laterally joined with the intervening strips of fascicular cambium. In this way, a continuous cylinder of dividing cambial tissue is produced.

There is great need for extensive investigations of the nature of the "transition" from primary to secondary growth in the vascular cylinder of stems. Sterling (1946, p. 43), on the basis of a study of vascularization in *Sequoia*, states that the original "eumeristem" cell of the shoot apex differentiates "so gradually to the mature cambial initial that there can be considerable difficulty in drawing a dividing line between procambium and cambium which would not appear artificial." It may also be doubted whether a significant distinction can be drawn between fascicular and interfascicular cambia. On the contrary, the *time* of appearance of the latter in stem ontogeny varies widely. In some plants, the interfascicular cambium becomes evident even before the primary phloem and primary xylem have completed their development in the vascular bundles. At the opposite extreme is the situation presented by such highly specialized dicotyledonous vines as *Aristolochia* and *Clematis*. Here the *time of appearance* of the interfascicular cambium is delayed, and this meristem arises by the tangential division of the large cells of the medullary rays between the vascular bundles (cf. Eames and MacDaniels, 1947, pp. 302-304). Many intergradations occur between these extremes, as is clearly revealed by the tabular summary given by Esau (1943a, p. 182). From a phylogenetic standpoint, the primitive type of vascular cylinder in the dicotyledons consists of a more or less continuous outer zone of secondary phloem enclosing a cylinder of secondary and primary xylem. From this type, as the result of progressive reduction in the duration of cambial activity and the eventual restriction of secondary tissues to the vascular bundles, the various types of dissected cylinders (largely primary in structure) have evolved (for further details, cf. Eames and MacDaniels, 1947, pp. 296-304).

(*b*) *The cambium of monocotyledons.* As a class, the monocotyledons are commonly described as devoid of cambial activity. This statement is true for many herbaceous types, as for example

the grasses. In these plants, the vascular system of the stem is wholly primary and consists of numerous vascular bundles distributed in a variety of patterns in the fundamental tissue system. Usually the bundles are of the *collateral type*, i.e., they consist of an external strip of primary phloem lying in contact with the primary xylem (cf. Esau's 1943c, detailed histogenetic study of the vascular bundle of *Zea*). The collateral bundles in many monocotyledonous stems are often accompanied by massive "external caps" or sheaths of fibers. In some monocotyledons (e.g., *Acorus*, and *Cordyline*) the bundles are designated as *amphivasal;* in this type a central strand of phloem is surrounded by a sheath of primary xylem.

In a detailed review of the literature, Esau (1943a, pp. 183-188) has discussed the numerous investigations dealing with the question of the cambium and secondary vascular tissues in certain monocotyledonous stems. As she points out "the terminology regarding the meristems involved in the growth of the monocotyledonous axis, however, is in need of revision for which a sure basis is still lacking." A good example of the difficulties of interpretation and nomenclature is provided by Ball's (1941) study of the shoot apex and cambial-like meristem of several palms. According to Ball, the growth of the stem in the species investigated results from the activity of two meristems, viz.: (1) the shoot apex, which contributes a narrow central column of parenchyma and a few vascular strands, and (2) the "*primary thickening meristem,*" which gives rise to the bulk of the stem tissues, i.e., the fundamental parenchyma and most of the vascular bundles. The expression "primary thickening meristem" is applied by Ball to a cambial-like zone of cells that originates from the "ground tissue" *beneath* the surface attachment of the young leaves. Since the relative degree of activity of the primary thickening meristem increases proportionally to the distance away from the shoot apex, the result is that the latter "is in the bottom of a bowl-shaped cavity during most of the post-seedling life of these palms" (cf. Ball, 1941, p. 826, Fig. 15). With respect to the progressive development of new vascular strands in the subterminal region of the stem, Ball found that the majority of them "originate in a centrifugal manner from the

bowl-shaped primary thickening meristem." In terms of structure, Ball states that the cambium-like meristem in *Phoenix* is devoid of distinguishable "initials" and because of the tiered arrangement of the cells resembles the cambia of such woody monocotyledons as *Cordyline, Dracaena, Dasylirion, Nolina,* and *Agave.* Whether the primary thickening meristem of palms and the "cambia" of other monocotyledons are strictly comparable with the vascular cambia of gymnosperms and dicotyledons is open to question and further study. In the two latter groups the cambium normally adds increments of phloem and xylem to a vascular cylinder. But in the monocotyledons under discussion, new *secondary vascular bundles,* separated by parenchyma are produced inwardly from the cambium and contribute to the growth in thickness of the stem. (For details regarding the ontogeny of "secondary bundles" in woody monocotyledons, cf. Chamberlain, 1921, and Cheadle, 1937.)

2. *The phellogen.* The early phases in development of secondary vascular tissues in woody stems are usually accompanied by the formation of *periderm* beneath the epidermis. Functionally, the periderm acts as a protective layer, replacing in this respect the epidermis that is eventually killed and sloughed away. Structurally, the term "periderm" is applied to the *phellogen* or cork cambium and its two derivative tissues, viz.: cork, or *phellem,* and *phelloderm.* The first formed phellogen in the stem appears to arise as a result of the growth and tangential division of epidermal, cortical, or phloem parenchyma cells. In some species, these first tangential divisions appear in the epidermis. Most commonly, perhaps, the phellogen originates in the outermost cells of the cortex; in *Vitis,* the first formed phellogen layer originates by the division of parenchyma cells in the metaphloem (Esau, 1948). There is evidence that in some stems the cortical phellogen first makes its appearance beneath the stomata, at which points *lenticels* are produced. Continued spread of the phellogen from these structures may result in the formation of a cylinder of cork cambium. As a result of the repeated tangential division of the phellogen cells, the derivative tissues exhibit alignment of the cells in radial rows. Cells differentiating toward the outside of the phellogen lose their protoplasts,

acquire *suberin* in their unpitted walls, and finally mature as cork cells. Cork cells are compactly arranged without intercellular spaces and may appear either empty or provided with "tannin-like" inclusions. The phelloderm tissue, which is usually much less in extent than the cork, originates from the inner derivatives of the phellogen. *Phelloderm cells* are decribed as being parenchyma-like in retaining their protoplasts and in having simple "pits" in their cellulose walls; in some plants, sclereids arise in the phelloderm tissue. The *functional life* of the first-formed phellogen in woody stems is short, and new layers of cork cambium arise successively from deeper regions of the cortex and primary phloem until, finally, the living cells of the secondary phloem participate in periderm formation. The ultimate result is the production in many species of shell-shaped layers of periderm that enclose masses of dead or dying cortical and phloem tissue. The term *rhytidome* has been applied to such exfoliating layers of tissue.

The first formed as well as later developed periderm layers in stems are usually provided with aerating structures termed *lenticels*. As stated above, lenticels are usually initiated by the appearance of a phellogen beneath a stoma (cf. Eames and MacDaniels, 1947, pp. 262-266). In the development of a lenticel, the phellogen, instead of producing typical cork, forms a mass of loosely arranged cells with unsuberized walls that make up the *complementary tissue*. This tissue in many lenticels may be subdivided by layers of smaller more compact cells termed *closing layers*. The pressure exerted by the outwardly developed mass of complementary tissue is sufficient to rupture the epidermis, which, together with the underlying layers of adjacent cork, curl back from the edges of the lenticel as flaps of broken tissue. In many plants (e.g., *Sambucus*), the extruded complementary tissue is very prominent. According to De Bary (1884, p. 561), the puffy swelling of lenticels in trees during wet weather may be the result of the "hygroscopicity" of the complementary tissue. As new layers of phellogen are initiated in successively deeper regions of the stem, a corresponding development of new lenticels occurs. These originate at the surface of the living tissue lining the cracks and furrows of the "bark."

V. Material for the Study of the Stem

The choice of material for the study of stem anatomy will naturally depend upon the forms available locally or upon the kinds of slides that can be secured from supply houses. Free-hand or CO_2-microtomed sections of stems stained with phloroglucinol-hydrochloric acid have proved useful and can be recommended for class study. But for the finer details of structure and development, permanent mounts of critically stained sections are necessary.

Prior to a survey of various stem types, the student should carefully investigate *serial* trans- and longisections through the terminal portion of a growing shoot, noting the following: the origin and structure of the *procambial strands* near the shoot apex; the position and structure of the *protophloem* and *protoxylem* in young vascular bundles in the stem and leaves; the structure and development of the epidermis, cortex, vascular cylinder, and pith at successive transverse levels distal to the shoot apex. Any of the following plants provide useful material for this fundamental study: *Sambucus, Helianthus, Coleus, Linum.*

1. *The stem of Pelargonium.* Examine a *transverse section* of the stem, and study the following tissues and regions from the edge of the section inwardly, viz.:

(*a*) The *epidermis,* a uniseriate layer of small oval or elliptical cells with thick inner and outer walls (the latter covered with a thin *cuticle*) and somewhat thinner radial walls. A protoplast, which may appear somewhat collapsed, should be evident in most cells. Certain of the protoderm cells have given rise to *stomata,* whereas others have developed to various types of trichomes, including glandular capitate hairs and multicellular unbranched hairs, the latter similar to the hairs already studied in the geranium leaf. (Refer to Exercise V.)

(*b*) Depending largely on the distance from the shoot apex at which the sections were taken, the early or later stages in the development of *cork,* or *phellem,* will be found. The *phellogen* in *Pelargonium* originates from tangential divisions in the subepidermal layer of cortical parenchyma cells. In studying the phellem in the stem of the geranium, notice that *druses* are

occasionally found in some of the inner cork cells. If the sections show a phellem four or five layers in thickness, note that the tangential (and to some extent the radial walls) are somewhat wavy or irregular. This condition, very commonly found in corky tissues, results from the constant pressure of the successively developing and enlarging cork cells upon the older cells of the phellem. *Phelloderm,* consisting of a few layers of living cells, is produced after a well-defined zone of cork has developed.

Internal to the periderm is found the *cortex,* a region composed of twelve or more layers of parenchyma cells separated from one another by prominent intercellular air spaces. A protoplast is present in many of these cortical cells, the main functions of which are photosynthesis and food storage, as is evidenced by the frequency of *starch grains* in these cells; *druses* of calcium oxylate are found in many of the cortical cells, occupying nearly the whole cavity of the cell.

(*c*) In direct contact with the innermost layer of cortical parenchyma cells occurs an unbroken cylinder of *bast fibers,* followed by several layers of small, thin-walled cells. Whether these fibers in *Pelargonium* originate entirely independently of the protophloem region of the vascular cylinder is a question to be solved only by a thorough investigation of stem ontogeny.

(*d*) The *vascular cylinder* in the young terminal region of the *Pelargonium* stem consists of typical *collateral bundles* separated from one another by strips of thin-walled parenchymatous tissue. These interfascicular portions of the vascular cylinder may be descriptively termed "medullary rays."

In view of the obvious variation in the size and degree of development of the vascular bundles in any given section, the following description is only general in nature, and all essential variations and details must be individually interpreted.

A well-developed *vascular bundle* in the geranium stem is somewhat wedge-shaped or triangular in cross section and consists of an external region of phloem tissue separated from the internal region of xylem tissue by the *cambial zone* in which cell divisions occur predominantly in the *tangential plane.*

Beginning with the phloem tissue of the bundle, note the *somewhat crushed primary phloem* lying directly against the

parenchyma described under (*c*) above; structurally, primary phloem in the geranium stem appears to consist of small sieve-tubes, companion cells, and phloem parenchyma. Lying directly internal to the primary phloem occurs the *secondary phloem*, which is composed of (1) *sieve-tubes*, rather large polygonal cells, apparently devoid of contents; (2) *companion cells*, extremely small, more or less *triangular cells closely joined* to the sieve-tubes and usually containing a definite nucleated protoplast; and (3) large, thin-walled *parenchyma cells*.

Separated from the secondary phloem by the "cambial zone" occurs the xylem tissue of the vascular bundle. In all the larger bundles, the xylem is of two kinds, viz.: (1) *secondary xylem*, an external layer of thick-walled, isodiametric, tightly joined cells arranged in more or less definite radial rows; and (2) the *primary xylem*, an internal group of rather large, more or less polygonal cells, irregularly arranged and embedded among isodiametric, thin-walled parenchyma cells. The smallest cells of the primary xylem (which has differentiated centrifugally from the procambial strand) are found nearest the *inner edge* of the vascular bundle and represent the first formed elements of the *protoxylem*. Notice particularly that in many protoxylem cells portions of the spiral thickening have been torn from the wall in the process of sectioning; in other cells, a piece of the spiral band may be seen projecting into the lumen of the cell. From what has already been said of the structural characteristics of the tracheary cells of the xylem (cf. Exercise X, p. 125), it is almost unnecessary to state that the limits between secondary xylem and metaxylem are virtually impossible to determine, especially when considering only transverse sections of a vascular bundle. In the interval between each of the individual bundles in the ring, there occurs a strip of small, thin-walled, obviously meristematic cells that are *continuous* with the cambial zone in the bundles themselves and represent what is termed the *interfascicular cambium*. The interfascicular cambium originates by the tangential division of cells in the medullary rays adjacent to the strips of fascicular *cambium*. In certain plants, the interfascicular cambium only forms parenchyma (e.g., *Clematis*, *Aristolochia*), but in *Pelargonium*, as in many herbaceous

plants, the interfascicular cambium gives rise to additional "bundles" composed entirely of secondary phloem and secondary xylem; the extensive development of vascular tissue from the interfascicular cambium usually results in the formation of a *complete cylinder of secondary xylem and phloem.* In studying the sections note that phloem is the first vascular tissue to differentiate from the interfascicular cambium and is later followed by the centrifugal formation of secondary xylem. Sterling (1946, p. 40) states with reference to *Sequoia:* "At first there are no xylem derivatives of the interfascicular cambium. However, sieve cells are formed which are quite similar (in transection) to the phloem produced by the fascicular cambium." It is probable that the appearance of sieve-tubes *before* tracheary elements in interfascicular areas is a common phenomenon in the stems of seed plants (cf. also Esau, 1945, p. 23, Figs. 18-19).

The center of the stem is occupied by the *pith,* which is composed exclusively of large, thin-walled *parenchyma cells* separated from one another by conspicuous intercellular airspaces. The extreme abundance of *starch grains* in the cells of the pith indicates that this region has as its function the storing of reserve food material.

2. *The stem of Tilia.* Obtain a stained slide with transverse, radial, and tangential sections of the stem. Examine and study the following tissues and regions from the periphery of the stem to its center, viz.:

(*a*) The *epidermis,* a uniseriate layer of cells that appears broken and cracked in numerous places because of the development of a prominent *periderm* beneath it. Structurally the cells of the epidermis are rather small, are oval in shape (in transectional view), and possess thick, slightly stratified outer walls overlaid by a *cuticle;* in most instances, ergastic materials and the remains of the disintegrated protoplast are present in the epidermal cells.

(*b*) Immediately within the epidermis occurs the *periderm* composed of three layers of tissue, viz.:

(1) The *phellem,* or cork, which is differentiated centrifugally from the phellogen and consists of a varying number of layers of narrow, laterally compressed cells arranged in definite

radial rows and densely packed (except the two outermost layers) with dark brown material that consists probably of substances classed under the general head of *tannins*.

(2) The *phellogen*, which is a uniseriate layer of meristematic cells found next to the innermost layer of phellem cells. In *Tilia*, as in so many woody stems, the phellogen is initiated by the tangential division of the outermost layer of cortical cells.

(3) Internal and directly next to the phellogen occur several layers of cells characterized in this instance by their "rectangular" form and obvious protoplasts. These layers of cells represent the *phelloderm* and differentiate centrifugally from the phellogen.

Note that the corky layer of the stem is definitely broken at certain points that appear as somewhat lens-shaped areas; these regions are known as *lenticels*. In studying the structure of the lenticels of the basswood stem, observe particularly the continuity between the phellogen and phelloderm of the lenticel and these tissues as they occur at either side of the lenticel. (*Note:* A supplementary study of lenticel structure and development can appropriately be made at this point. The lenticels of *Sambucus* and *Prunus* are recommended.)

(*c*) Directly internal to the periderm occurs the *cortex*, which consists of two rather definite regions, viz.:

(1) An *outer region* composed of four or more layers of collenchyma cells, which in the position of their thickened primary walls appear intermediate in character between "angular" and "plate" collenchyma. (Cf. Exercise VII, p. 87.) Notice that intercellular air spaces are extremely small and difficult to distinguish.

(2) An *inner region* composed of *parenchyma cells* with active protoplasts and cells containing druses of *calcium oxalate*. The latter type of cells tend to occur in vertical superposed groups (refer to the radial section of the stem). It is important to notice the crushed appearance of many of the parenchyma cells in the cortex; this condition has been caused by the pressure of the secondary phloem, which is constantly pushed toward the periphery of the stem.

(3) *The vascular cylinder*. The phloem occurs just within

the innermost layer of the cortex and consists of a cylinder made up of wedges of tissue showing a characteristic "banded" appearance that alternates with triangular sectors "homogeneous" in structure; the wedges showing the band-like structure are broadest next to the cambium, whereas the reverse relationship exists in the homogeneous sectors. A definable "pericycle" is absent in *Tilia* and hence the boundary of the vascular cylinder is represented by the *protophloem fibers.*

Examine in detail, first of all, the structure of one of the large *"banded" sectors,* observing that the characteristic "banded" appearance of such a sector is due to the alternation of tangential layers of extremely thick-walled *fibers* with layers of thinner-walled cells. These thick-walled *phloem fibers,* which provide mechanical support and flexibility to the stem, have long been known as "bast fibers," and their prominent development in the genus *Tilia* is responsible for the old English name of this tree, i.e., "bast-wood," which in American usage has been changed to "basswood." Under high power, the phloem or bast fibers appear irregularly polyhedral, are closely joined without evident intercellular air spaces, and possess extremely small lumina; under especially favorable conditions of magnification and illumination the infrequent canal-like pits between adjacent cells may be visible (for a detailed discussion of bast fibers cf. Exercise IX, pp. 107-111).

The layers of thinner-walled cells alternating with the bands of bast fibers are composed of the conducting and storage cells of the phloem, viz.: radial rows (one or occasionally two cells in thickness) of living parenchyma cells formed from the cambium and extending out to the cortex through the phloem; these radial rows of cells (which, of course, are actually sheets of tissue) are *phloem rays* and are directly continuous across the cambium with corresponding rays of the secondary xylem. The rays function both in radial transportation as well as in the storage of food materials. Starch grains are often present in these cells. The *sieve-tubes,* the important vertical conducting elements of the phloem, appear in transverse section as large, somewhat irregular, thin-walled cells which appear devoid of protoplasts and are closely joined on their smaller wall-facets with the *com-*

panion cells. The latter are very small, appear more or less triangular in transverse section, and in most cases possess definite nucleated protoplasts. Interspersed among the sieve-tubes are the small, isodiametric *phloem parenchyma cells*, which possess a living protoplast.

Turning now to the wedges of *"homogeneous tissue,"* it will be found that these sectors are entirely composed of somewhat "rectangular" parenchyma cells, most of which are provided with a definite protoplast. The characteristic form of these sectors is due to a *process of dilatation* of the multiseriate phloem rays lying between the "banded" sectors of the stem. In other words, certain of the phloem rays (usually those that are two or three cells in width at the cambium) increase continuously in width by the *radial division* of the parenchyma cells; the intervening areas of phloem (i.e., those containing the bast fibers and the sieve-tubes) are thus separated from each other and correspond in position to the phloem portions of the original vascular bundles. As the stem increases in age, a similar *process of dilatation* occurs in the smaller rays of the phloem in the "banded sectors" with the result that the latter become successively subdivided into smaller groups (for complete details, cf. De Bary, 1884, pp. 536-537). It should be noted that these *dilated phloem rays* are directly continuous across the cambium with bi- or triseriate *xylem rays*. Druses occasionally appear in the parenchyma cells of the dilated phloem rays.

The *cambial zone* occurs directly between the secondary xylem and the secondary phloem and consists of the *uniseriate cambium* itself and a varying number of layers of "maturing" vascular elements. A careful study of the "cambial zone" will help in understanding the direction of formation of secondary xylem and secondary phloem and, furthermore, will shed some light on the nature of the early developmental stages of the cells making up these tissues.

Within the "cambial zone" occurs the cylinder of *secondary xylem*, which is composed of two or three more or less concentric layers or cylinders of xylem tissue, each of which is known as an *annual ring*. In all woody stems of north temperate plants, an annual ring represents the amount of secondary xylem formed

during a single growing season. The presence of annual rings in the secondary xylem of perennial woody plants seems to be determined to some extent by seasonal conditions, since the xylem tissue formed in the spring (the so-called *early wood*) differs somewhat in respect to the size, type, and arrangement of its cells from that formed in the summer (the so-called *late wood*). It is because of this structural difference between early and late wood that an annual ring appears distinct. In many cases, the cells formed during late summer tend to be somewhat smaller and thicker-walled than those arising in the spring; often the difference is emphasized by the localization of the majority of the vessels in the early wood. Hence, there is usually a clear boundary between the last formed xylem of a given growth period and the first formed xylem of the next. (For further details on annual rings, cf. Eames and MacDaniels, 1947, pp. 208-214.)

The secondary xylem of *Tilia* is, like that of many woody dicotyledons, histologically complex and is formed of the following types of cells:

(*a*) *Vessel elements,* which are large prominent cells, rather polygonal in transverse section, and possessing large empty lumina. In *Tilia,* the vessels are rather uniformly scattered throughout both the early and late wood; for this reason, the wood is termed *diffuse porous* (cf. Brown and Panshin, 1940, pp. 503-504). In especially thin regions of the section, small bordered pit-pairs may be seen in cross-sectional view. Careful focusing should reveal the "compound middle lamella."

(*b*) *Tracheids,* which are smaller in size than the vessels and often more or less rectangular in shape as seen in transverse section. In contrast to the vessels, the tracheids are frequently arranged in definite radial rows.

(*c*) *Fibers,* which are somewhat irregular in shape and are usually much smaller than either the vessels or tracheids and are usually provided with thicker walls.

(*d*) Scattered among the vessels, tracheids, and fibers, occur the *wood parenchyma cells,* which are small in size, "isodiametric" in form, and possess a definite protoplast. It should be

realized that wood parenchyma cells occur in "vertical chains" or rows and function primarily in the storage of certain carbohydrates, particularly starch; the function of wood parenchyma in "assisting" the translocation of substances in the xylem is imperfectly understood. In *Tilia*, the vertical strands of wood parenchyma are diffuse in distribution within each ring, i.e., without definite relation to the vessels. This type of wood parenchyma distribution is termed "apotracheal." (For a thorough discussion of the problems of terminology of wood parenchyma, cf. Bailey and Howard, 1941b.)

Extending radially through the secondary xylem are the *rays*, which are of two types, viz.:

(1) *Large rays*, which represent the xylem "extension" of the large specialized dilated rays of the phloem discussed previously. In the second annual ring, these large rays may be two or more cells in width but, at least in the inner portion of the first annual ring, they become a single cell in width and finally terminate directly in the pith. Such rays have been termed "primary medullary rays."

(2) *Small rays*, one cell in width, which are the xylem extension of the rays extending through the "banded sectors" of the phloem.

The *primary xylem* occurs next to the pith and is completely surrounded externally by the cylinder of secondary xylem. It is naturally very difficult to distinguish between the *metaxylem* and the first formed elements of the secondary xylem in a transverse section of the stem of *Tilia*. The *protoxylem*, however, is quite distinct and appears as solitary or grouped thick-walled cells embedded among the small parenchyma cells of the primary xylem at the periphery of the pith; many protoxylem elements become crushed and excessively stretched during the phase of elongation growth in the stem. The *pith* is a solid rod of tissue occupying the center of the stem. In contrast to the condition in the geranium stem, the pith of *Tilia* is not homogeneous but shows a certain amount of cell specialization as follows:

The *periphery of the pith* is formed of several layers of rather thick-walled parenchyma cells that are provided with protoplasts and in addition contain very small starch grains; others of these peripheral cells are filled with dark-staining granular material,

the exact nature of which is obscure at present. The bulk of the pith is composed of much larger parenchyma cells separated by definite intercellular air-spaces; some of these cells likewise contain small starch grains and protoplasts. Interspersed among these large pith cells occur much smaller thicker-walled parenchyma cells filled with dark-staining granular bodies; these latter cells are found singly or in groups but in longisection appear in scattered vertical series.

Attention must finally be directed to the *mucilage cells*, which are arranged in a more or less definite "ring" near the periphery of the pith. These *mucilage cells* may be identified by their large size, by the disorganized reddish or purple material found in them, and by the jacket of starch-containing parenchyma cells that surrounds each of them. According to the detailed investigations of Walliczek (1893, pp. 247-253), the mucilage cells in *Tilia grandiflora* first appear in the young pith a short distance behind the shoot apex; mucilage cells are also later developed in the cortex and in the "bark" of the stem. Walliczek found that very often the mucilage cells occur in groups that later, as the result of the partial or complete chemical transformation of the original walls, fuse to form large *mucilage cavities*. The mucilage in mature cells or cavities usually appears as a series of conspicious lamellae.

3. *The stem of Aristolochia.* This stem has its vascular system in the form of a "ring" of bundles separated from each other by broad *medullary rays* composed of parenchyma cells. These radially directed sheets of parenchyma cells may extend vertically the length of an internode or more and in many cases continue to grow (by means of an interfascicular cambium) at the same rate as the fascicular cambium that is increasing the size of the vascular bundle.

To understand the structure and development of this highly specialized type of vascular cylinder, a study should be made of a series of transections, beginning with the extreme terminal region of the shoot and extending to internodes several years in age. Examine *first* a transection of a very young portion of the stem, noting the following tissues and regions in progressing from the edge of the section to its center, viz.:

(*a*) A typical *uniseriate epidermis* composed of rather

densely protoplasmic cells. Note the extremely thick *cuticle* that covers the epidermis.

(*b*) Internal to the epidermis occurs the *cortex*, composed of an outer zone of rather thin-walled "angular" *collenchyma* cells and an inner zone of large "isodiametric" *parenchyma* cells; note the presence of large *druses* in many of the parenchyma cells of the cortex.

(*c*) The *vascular cylinder* is sharply delimited by a broad cylinder of closely joined *fibers*, the secondary walls of which are still increasing in thickness. Internal to the fibers occurs *parenchyma*, which is quite similar in general structure to the cortical parenchyma. As in *Pelargonium*, it is improbable that these zones of sclerenchyma and parenchyma collectively represent a "pericycle." However, it is entirely possible that the sclerenchyma cells may originate external to the protophloem.

(*d*) The *vascular system* proper consists of a cylinder of typical *collateral bundles* in each of which the clearly distinct phloem and xylem is separated by a cambial zone (the *fascicular cambium*). Note particularly the crushed or obliterated *protophloem* at the outer edge of each bundle.

The *pith* of the stem is large and is composed of parenchyma cells in many of which *druses* are evident.

Early stages in the development of the *interfascicular cambium* should be evident also in this section or at slightly lower levels in the stem.

Next obtain a transverse section of a two- or three-year-old stem and notice the *profound changes* in structure that have been occasioned by *secondary growth*, viz.:

(*a*) *A discontinuous* and rather thick layer of *phellem* (cork) has appeared as the result of the activity of the phellogen. Notice particularly how the epidermis has been forcibly broken away from the cortex; strips of epidermal tissue are visible on the outer surface of the corky tissue. A comparatively extensive development of *phelloderm* can also be seen internal to the phellogen. *Lenticels* are well developed at certain points.

(*b*) The previously *continuous* cylinder of *collenchyma* has been broken as the result of the pressure of secondary growth. Notice that parenchyma cells of the cortex have intruded into the gaps between the strips of collenchyma cells.

(*c*) The *previously continuous* cylinder of *fibers* has likewise been ruptured, and the gaps filled by neighboring parenchyma cells. Notice the beginnings of wall thickening and lignification in some of the parenchyma cells between the strips of fibers; these parenchyma cells will finally become thick-walled *brachysclereids* (by a process of secondary sclerosis) and will thus effectively "repair" the broken mechanical cylinder. According to Schellenberg (1899, p. 311), the rupture and "repair" of the sclerenchyma cylinder may occur repeatedly during the early growth in thickness of the stem in *Aristolochia Sipho.*

(*d*) The *medullary rays* between the vascular bundles have become broad and long and their component parenchyma cells are arranged in more or less definite radial rows as the result of the continued activity of the *interfascicular cambium.*

(*e*) Each *vascular bundle* has increased enormously in size as the result of the continued activity of the *fascicular cambium.* Notice the *crushed condition* of the cells in the outer portion of the *phloem;* this has resulted from the outward expansion of the secondary phloem. The *xylem portion* of each vascular bundle shows 2-3 definite *annual* increments of growths; notice that the largest vessels occur at the edge (i.e., in the "early wood") of each annual increment.

(*f*) The irregularly shaped *pith* is now much reduced in extent and shows clear indications of crushing. *Aristolochia* represents one of the exceptional cases where the pith is actually compressed as the result of secondary growth. According to Schellenberg (1899, p. 313) this compression usually occurs before the rupture of the sclerenchyma cylinder external to the vascular bundles.

4. *The stem of monocotyledons.* The stem in many of the monocotyledons is characterized by the fact that the numerous vascular bundles are more or less scattered in various patterns through a part or all of the fundamental tissue system. In *Zea,* for example, scattered vascular bundles occur throughout the entire fundamental system, whereas in *Triticum* the vascular bundles (in a staggered, double series) are restricted to the outer hypodermal region and the mature stem is hollow. In some genera of the grasses, a single, cylindrically arranged series of bundles occurs. Monocotyledonous bundles lack a cambium and

are thus wholly primary in structure (cf., for example, Esau's 1943c study on *Zea*). *Sclerenchyma* is well developed in many monocotyledonous stems, occurring both as caps or massive sheaths in contact with the bundles and as a well-defined cylinder beneath the epidermis.

Obtain a transverse section of the stem of *Zea* and note under low power the characteristic arrangement of the *collateral vascular bundles* that are smaller and more numerous near the periphery of the stem than in the center. Study under high power the structure of a mature bundle observing (1) the well-developed sclerenchymatous *bundle sheath,* (2) the vestiges of the crushed and obliterated *protophloem,* (3) the well-defined *metaphloem* of sieve-tubes and companion cells, and (4) the prominent *lacuna* at the inner edge of the xylem. This lacuna results from the complete destruction of the *protoxylem vessels* during stem elongation (cf. Esau, 1943c, pp. 339-341). The metaxylem consists of tracheary elements, the most prominent of which are three very large vessels.

For comparative purposes, study a transection of the stem of *Triticum,* noting the distinctive pattern of arrangement of (1) the vertical bands of photosynthetic cortical parenchyma, (2) the hypodermal sclerenchyma, and (3) the double series of vascular bundles that closely resemble those of *Zea* in their histology (cf. Hayward, 1938, pp. 163-165).

In order to study *secondary growth* in woody monocotyledons, secure trans- and longisections of portions of the outer region of old stems of *Yucca* and *Cordyline.* In both genera, the cambium arises within the parenchyma tissue external to the "primary" bundles. Note especially the origin of strands of "procambium-like" cells from the subdivision of certain of the cambial cells. These strands give rise respectively to the *collateral* and the *amphivasal* "secondary bundles" of *Yucca* and *Cordyline.*

5. *The stem of conifers* (e.g., *Pinus*) is anatomically similar to that of many woody dicotyledons in that the activity of a vascular cambium forms continuous cylinders of secondary phloem and secondary xylem. Obtain tran- and longisections of young

stems of *Pinus* noting in particular the following interesting histological features, viz.:

(*a*) *Resin canals.* These structures occur in both the cortex as well as the secondary xylem. A mature resin canal consists of an extensive tube-like *cavity* (produced by the separation of a series of originally connected cells) bordered by an *epithelium* composed of cells which excrete the resinous material into the cavity. In the case of the secondary xylem of *Pinus*, resin canals are oriented *longitudinally*, i.e., scattered within the "*matrix*" of tracheids, and *transversely*, i.e., extending radially in the center of many of the *xylem rays* (cf. Brown and Panshin, 1940, p. 132, Fig. 29). In both types, the epithelial cells originate as derivatives of the cambial initials. The cortical resin canals develop by the separation of vertical series of parenchymatous elements in the maturing ground meristem.

(*b*) The *secondary xylem*, aside from the resin canals just described, consists of fibers, fiber-tracheids and uniseriate xylem rays. Vertical wood parenchyma is absent from the secondary xylem of *Pinus* but is abundantly developed in many other coniferous genera (for illustrations of the structure of the stem in *Pinus* and other gymnosperms cf. Jeffrey, 1917).

6. *Nodal anatomy of dicotyledonous stems.* With the use of a razor blade or sliding microtome, cut *serial transections* through the nodal region of living stems, arranging the sections in proper order on a slide and flooding them with *phloroglucinol-hydrochloric acid.* Wash off the reagent (after the xylem appears a bright red in color), add water and examine with the aid of a binocular dissecting microscope. Note the *leaf traces* and their associated *gaps* and the relation of the vascular system of the *bud* to the vascular system of the main axis. Material illustrating the various types of nodal anatomy can readily be selected from the tabular summary given by Sinnott (1914, pp. 319-320).

VI. Suggested Drawings and Notes

1. Prepare a large diagrammatic drawing of the transection of the stem of *Pelargonium*, indicating by legends and labels the position and extent of all the tissues and regions studied.

2. Prepare a drawing, similar to the above, based upon the study of the stem of *Tilia*.

3. Prepare a series of diagrammatic drawings of transections of the stem of *Aristolochia*. These diagrams should illustrate the effects of secondary growth on the primary structure of the stem.

4. Make a detailed cellular drawing of the periderm of the stem of *Pelargonium*, *Tilia* or *Aristolochia* showing the arrangement and structure of the phellen, phellogen, and phelloderm.

5. Draw the structure of a lenticel (of *Sambucus* or *Prunus*) as seen in median longisectional view, showing the complementary tissue, the closing layers, the phellogen, and the relation of these tissues to the adjacent periderm.

6. Prepare diagrams, showing accurately the position of tissues and vascular bundles as seen in the transections of the stems of *Zea* and *Triticum*.

7. Show by means of diagrams the position of the cambium and the method of origin of the secondary vascular bundles in the stem of *Cordyline* or *Yucca*. Outline on a large scale a single, mature bundle in each genus, as seen in transectional view, filling in the cellular details of a small portion of the fibers, metaphloem, and primary xylem.

8. Draw in detail the resin canals in the secondary xylem of *Pinus*, showing (*a*) the origin of the epithelial tissue from the vascular cambium, (*b*) the mature structure of the longitudinal and transverse resin canals.

9. Prepare diagrams to illustrate the various types of nodal anatomy studied.

10. Prepare a brief outline of the changes that occur during the development of "heartwood" in the stems of aborescent seed plants (cf. Eames and MacDaniels, 1947, pp. 220-224; Brown and Panshin, 1940, pp. 35-39).

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Exercise XIV

THE LEAF

As stated in the previous exercise, it is difficult, on both theoretical as well as practical grounds, to demarcate the leaf from the stem. If the widely held belief is true that the leaves of the Pteropsida have arisen phylogenetically from determinate branch systems, this difficulty becomes understandable. As a matter of fact, the vascular anatomy of the *leaf axis* (i.e., the petiole-midrib region) is often extremely complex and stem-like. Indeed perhaps the most consistent character that distinguishes the leaf from the stem, aside from its prevailingly dorsi-ventral symmetry, is the *early cessation of apical growth.* The leaves of many ferns retain an active apical meristem for a relatively long period in ontogeny, but in seed plants true apical growth ceases at an extremely early period and the ultimate size of the leaf is determined by subapical and submarginal development. From a morphological as well as an anatomical viewpoint, leaves are without question the most diversified structures produced by seed plants. (For detailed treatises dealing with the morphology, ontogeny, and comparative anatomy of leaves, cf. Goebel, 1905; Solereder, 1908; Arber, 1925; Troll, 1938, 1939a, 1939b, 1939c.) The foliage leaf, which is the most familiar type, varies from the small scale-like structures found in certain gymnosperms and angiosperms to the enormous and complex leaves of the palms. In addition to foliage leaves, other types of foliar organs are usually included under the morphological concept of leaf. As illustrations may be mentioned cotyledons, bud scales, bracts, and, according to classical theory, the appendages of the flower.

Because a large proportion of the voluminous data on the foliar anatomy of the dicotyledons still remain to be collated and re-examined (cf. Solereder, 1908), broad generalizations regarding leaf histology are out of the question at present. Consequently the brief résumé given below is intended merely as an introductory guide to certain of the salient trends of histological

specialization illustrated by the foliage leaves of the angiosperms. Students desiring information on the structure and development of leaves in the gymnosperms should consult the data and the literature given in the papers of Florin (1931), Chamberlain (1935), Cross (1940, 1941, 1942), and Johnson (1943).

I. Histology of the Foliage Leaf

1. *Epidermis.* The epidermis of the leaf varies considerably as to the occurrence and types of *trichomes* developed, the structure and arrangement of the *stomata,* and the number of layers of cells that compose it. (For a detailed treatment of these topics the student should review the text material presented in Exercise V.)

2. *Mesophyll.* This term is used in a very general sense to designate the thin-walled *parenchyma tissue* that often constitutes the bulk of the internal "fundamental tissue system" of leaves. The greatest diversity exists with respect to the forms of the parenchymatous cells of the mesophyll and their arrangement in relation to the veins and sclerenchyma in the leaf. (For a detailed treatment of this matter from a physiological point of view, cf. Haberlandt, 1914, Ch. VI.) In the leaves of some angiosperms (e.g., *Zea* and certain other grasses) the mesophyll is composed of more or less polygonal cells separated by small air spaces. But in many dicotyledons, two "forms" of parenchyma occur, viz.: *palisade parenchyma* composed of columnar cells, separated by intercellular spaces, arranged with their long axes at right angles to the epidermis, and (*b*) *spongy parenchyma,* made up of very loosely arranged lobed or stellately branched cells. In typical *dorsiventral* laminae, the palisade cells are developed as one or more layers of tissue directly beneath the adaxial epidermis, while in many *isolateral* leaves, such as the phyllodes of *Acacia* (Boke, 1940) and the "adult" leaves of *Eucalyptus,* palisade parenchyma occurs on both faces of the lamina. According to the résumé in Haberlandt (1914, pp. 293-297) the position of palisade tissue and the degree of its development are controlled, to some extent, by light intensity. *Chloroplasts* are abundant in the mesophyll, especially in the cells of the palisade parenchyma, and this tissue represents the region

of most intensive photosynthesis in the majority of angiosperms.

Various types of *idioblasts* are commonly encountered in the mesophyll of the leaf, among the most common of which are *secretory cells*, *lithocysts*, and *sclereids*. In the leaves of many dicotyledons, e.g., *Mouriria*, *Memecylon*, and *Boscia*, ramified or fiber-like idioblastic sclereids are extremely abundant (cf. Foster, 1946, 1947 and Pestalozzi, 1898).

3. *Vascular system.* As explained in the previous exercise (Exercise XIII, p. 156), the vascular supply of the leaf in dicotyledons consists of one, three, or many traces. These leaf traces may extend throughout the entire "leaf axis" (i.e., leaf base, petiole, midrib, or rhachis of lamina) as discrete collateral bundles with the phloem oriented toward the lower or abaxial surface. Very commonly, however, the primary leaf traces may either "subdivide" into a crescentic series of bundles or conversely may "fuse" to an arc-shaped strand. In many dicotyledons there occurs, in addition to the primary leaf-trace system, a series of "accessory" strands near the upper surface of the leaf axis. These strands join with the leaf traces at various levels and exhibit an inverse orientation of xylem and phloem, i.e., the latter is oriented towards the upper surface of the leaf axis (Eames and MacDaniels, 1947, p. 329, Figs. 152-153). Ontogenetically these inversely oriented accessory strands originate from a cambial-like meristem situated below the adaxial surface of the young leaf axis (Foster, 1935a, 1935b; Troll, 1938, pp. 1005-1006; 1939a, pp. 1149-1152). The vascular system of the *lamina wings* consists of an intricate series of interconnected bundles that collectively form the most diverse patterns or types of *venation*. The various forms of venation have been widely utilized in the classification of angiosperms and in the morphological interpretation of such organs as bracts, sepals, and petals (cf. Glück, 1919). In the laminae of many leaves, the numerous and extremely small veinlets end blindly in the mesophyll and are termed *vein endings*. Histologically, such vascular terminations often consist only of one or several tracheary elements surrounded by a jacket of *border parenchyma* (for a thorough study of border parenchyma in leaves cf. Armacost 1944). Vein endings are usually devoid of sieve-tube elements. Several interest-

ing and distinctive trends of specialization in vein endings of the leaves of certain dicotyledons deserve mention at this point. In some genera, the terminal tracheary elements are greatly enlarged, ovoid, or irregularly branched cells, which contrast strikingly with the slender prosenchymatous tracheids in the subterminal region of the veinlet; these peculiar cells are termed *"storage tracheids"* and may serve as water reservoirs (cf. Pirwitz, 1931). The vein endings in another series of recently studied genera are in direct contact with one or more *terminal sclereids;* these elements may occur singly or in groups and are lobed or branched in the most diverse manner (Foster, 1946, 1947). The phylogenetic origin of terminal "storage tracheids" and terminal sclereids, as well as the physiological roles of such cells, represent interesting but at present unsolved problems (cf. Foster, 1947).

The vascular bundles in most angiospermous leaves are surrounded by more or less conspicious "bundle sheaths." In the case of the small veinlets of the lamina, the bundle sheath may consist simply of a uniseriate jacket of enlarged, relatively thin-walled parenchyma cells containing large plastids; a good example is provided by the sheaths of the smaller veins in the leaf of *Zea* (for a detailed description of plastid structure and function in the cells of the bundle sheath of *Zea,* cf. Rhoades and Carvalho, 1944). Larger veins, both in the lamina wings, as well as in the petiole and midrib, are frequently jacketed by a sheath of sclerenchyma (Esau, 1943; Foster, 1947). In the grasses, which are notable for the development of both fascicular and extrafascicular sclerenchyma in the lamina, the thick-walled cells of the bundle sheath are an integral part of the vascular tissue, from an ontogenetic standpoint.

II. Foliage-leaf Differentiation in Angiosperms

In herbaceous annuals, the differentiation of a leaf from primordium to maturity is a continuous and uninterrupted process. But in woody dicotyledons of the north temperate zone, leaves are initiated and begin differentiation in one season, enter the winter period of bud-dormancy in various stages of development, and only complete their ontogeny the following spring. Within the limited scope of this book it is only possible to outline

and to compare briefly the most salient features of the successive steps in foliar histogenesis. For reviews and extensive comparative treatments of this subject reference should be made to Foster (1936), Troll (1938), Sifton (1944) and Majumdar (1945). The differentiation of an angiosperm leaf may be conveniently described under the following somewhat arbitrary "phases" or stages, viz.:

1. *Initiation.* The initiation of a leaf begins with the periclinal division of a small group of cells at the side of the shoot apex. Great variation, however, exists with respect to the layer or layers of the apex in which these early divisions first appear. Sharman (1942, 1945), for example, has found that in many grasses, the leaf is initiated by the periclinal division of cells *both* of the surface and subsurface layers of the apex. In this case, a considerable portion of the internal "ground meristem" of the young foliar primordium is derived from the *outermost* cell layer of the shoot apex. An essentially similar type of leaf initiation has also been reported for the bamboo, *Sinocalamus Beecheyana*, by Hsü (1944). On the contrary, in certain other monocotyledons (e.g., *Tulipa*, Sass, 1944) and apparently *in all investigated dicotyledons*, the surface tunica layer takes no part in the initiation of the internal tissue of the leaf. By means of continued anticlinal divisions, the cells of this layer adjust themselves to the growth of the emerging primordium and give rise to the protoderm of the young leaf. In this widespread type of leaf inception, the initiating periclinal divisions occur, to varying degrees, in the inner layers of the tunica as well as in the adjacent cells of the corpus. Indeed, the extent to which inner tunica and corpus cells contribute to the internal tissues of the foliage leaf primordium is highly variable, and no rigid or general scheme of development has been detected. This is illustrated by the recent study of foliar initiation in colchicine-induced periclinal chimeras in *Datura*. (For a discussion of the structure of the shoot apices in these chimeras cf. Exercise III, p. 36.) In this plant, the greater part of the future mesophyll of the foliage leaf, sepal, and petal originates from cells derived from the inner tunica layer, whereas the acropetally developing procambial strands arise from cells of the corpus. In the stamen,

however, the derivatives of the second tunica layer develop as a discrete hypodermal layer and the bulk of the inner tissue (including the procambium) of this organ originates from the corpus (cf. Satina and Blakeslee, 1941, p. 866, Figs. 11-26).

2. *Early differentiation.* Continued cell divisions soon result in the *emergence* of the leaf primordium from the shoot apex as a papillate or crescent-shaped structure. In addition to an external *protoderm layer* and an inner mass of *ground meristem,* the foliar primordium is provided with a median *procambial strand* which, as explained previously (Exercise XIII, p. 157), develops acropetally from the adjacent procambium of the shoot axis. This extremely early demarcation of primary meristematic tissues is characteristic of the foliar primordia of a wide range of angiosperms (Foster, 1935a; Satina and Blakeslee, 1941; Esau, 1942; Reeve, 1942; Engard, 1944).

3. *Development of the leaf axis.* In many dicotyledons, the formation of the thin, lateral, wing-like portions of the lamina or the development of lateral leaflets is preceded by the early differentiation of the leaf axis. As a result of rapid elongation, the foliar primordium gradually assumes the form of a tapering, adaxially flattened cone, the tip of which functions for a short time as an *apical meristem.* At an early period, however, in some plants when the leaf is less than 1 mm. in length, the cells at the leaf apex begin to show evidence of histological maturation, and all further growth in length results from cell division and cell elongation distal to the tip. Very commonly, the vertical extension of the leaf-axis is accompanied by a marked *increase in its radial thickness.* This is especially pronounced in the numerous cases where a cambial-like strip of tangentially dividing cells originates along the *adaxial face* of the primordium. To such a cambial-like zone of tissue the terms "adaxial meristem" or "ventral meristem" have been applied (Foster, 1935a, 1935b, 1936; Troll, 1938, pp. 1005-1009; Engard, 1944). As indicated earlier in this exercise, accessory vascular bundles may eventually differentiate from the cells of the adaxial meristem.

4. *Origin of the lamina.* During the early growth in length and thickness of the young leaf axis, its *adaxial margins* remain in a highly meristematic state as compared with the more internal

cells of the ground meristem. In the case of many simple leaves, the marginal regions, by means of intensified growth and cell division, give rise to two delicate ridges or wing-like extensions, from which the lamina is gradually differentiated. Except in sessile leaves, a short basal portion of the leaf axis fails to exhibit significant marginal growth; from this region, as a result of subsequent elongation, the *petiole* originates. As seen in transectional view, each half of the young, developing lamina consists of a continuous protoderm that encloses several layers of ground meristem. In the light of many investigations it is clear that for some time the addition of new cells to the various layers of the lamina proceeds from a series of vertically superposed *marginal* and *submarginal initials*. (For extended discussions of the varied types of marginal growth in the foliar organs of vascular plants, cf. Foster, 1936, 1937; Troll, 1938, pp. 998-1005.) Usually in the angiosperms, the marginal initials (i.e, the outermost cells at the edge of the young lamina) divide only in the anticlinal plane, thus contributing new cells to the adaxial and abaxial protoderm layers. But in the leaves of certain monocotyledons and in the bud scales of *Rhododendron* spp., more or less abundant periclinal divisions occur in the marginal initials, thus adding new cells to the adjacent ground meristem (Foster, 1937; Sharman, 1942, 1945). Investigation has shown recently that the planes of cell division in submarginal initials vary, not only between leaves of different genera but between the various foliar organs of the same species; from these divisions new cells are contributed to the internal cell layers of the young lamina. (For a contrast between marginal growth in bud scales and foliage leaves, cf. Foster, 1935a.)

In pinnately or palmately compound leaves, the lateral leaflets arise from the adaxial meristematic margins of the young leaf-axis as two series of papillae. Frequently, as in *Carya* (Foster, 1932, 1935a) the order of appearance of the lateral leaflet primordia is acropetal; in many dicotyledons, however, the lateral leaflets develop basipetally. (For a monographic treatment of the ontogeny of pinnate leaves, cf. Troll, 1939b, pp. 1426-1676.) In *Carya*, each of the lateral leaflet primordia first develops an axial region (comparable to the main axis of the

leaf) from the adaxial margins of which the wings of the leaflet develop. The tip of the main leaf axis in *Carya* becomes the terminal leaflet.

5. *Histogenesis and maturation of tissues in the lamina.* True marginal growth, although of apparently longer duration than apical growth, ceases relatively early and the further development of the lamina-halves is achieved by "surface growth," i.e., the predominantly *anticlinal division* of the various layers of the lamina. Because of the conspicuously *stratified character* of the various meristematic layers (which have been collectively termed *Plattenmeristeme* by Schüepp, 1926, p. 18), it proves relatively easy to trace the origin of the epidermis, the palisade parenchyma, and the spongy mesophyll; in many investigated dicotyledons, these tissues originate from specific layers in the young lamina (cf. Foster, 1936, Figs. 6-10). The regular stratification of cells, however, is disturbed to varying degrees by the progressive development of the veins and their associated sheaths and mechanical tissues, i.e., collenchyma or sclerenchyma; as a consequence, during the final phases of surface growth, the regular stratification of cells is restricted to those portions of the lamina *between* the major lateral veins (cf. Avery, 1933; Foster, 1935a). One of the last of the leaf tissues to cease growth and cell division is the palisade parenchyma; this region of the mesophyll may remain in a meristematic condition for some time after divisions have ceased in the epidermal layers and the spongy parenchyma (Foster, 1936; Tetley, 1936; MacDaniels and Cowart, 1944). Our present knowledge of the details of tissue maturation in the laminae of angiosperm leaves is very meagre. In particular, the nature of the physiological and morphological interrelationships between the epidermis, mesophyll, and the major and minor veins needs both extensive as well as intensive study. (For experimental work in the problem cf. Armacost 1944; Wylie 1938, 1943, 1946a, 1946b; and Plymale and Wylie 1944.)

III. Material for the Study of the Leaf

1. Obtain prepared tran- and longisections through the terminal buds of *Zea* and some available dicotyledon (e.g., *Linum*, *Nicotiana*, *Syringa vulgaris*) and study the initiation and early

phases of differentiation of the leaves. If a closely graded series of stages in leaf development is available, it will be possible to trace the origin of the epidermal layers, the mesophyll and the veins from the various meristematic layers of the young lamina. In the dicotyledonous buds, *paradermal sections* (i.e., sections cut in the plane of the epidermis) are particularly instructive in any study of the differentiation of the mesophyll and the vein endings.

2. With a sharp razor cut serial sections through the leaf base and petiole of fresh leaves of *Syringa*, *Populus*, *Carya* and *Acer*, and stain each series separately in phloroglucinol-hydrochloric acid. Study the stained sections (after washing off the reagent with water) under the dissecting binocular microscope, noting the varied patterns of the vascular system. The most comprehensive idea of the intricate vascular system of the lamina is obtained by using *cleared leaves;*[1] portions of the laminae of *Zea*, *Syringa*, and *Phaseolus* are recommended for this study.

3. *The lamina of the leaf of Syringa vulgaris.* Obtain a stained transection of the lamina and study its histology under low magnification. Note first the clear distinction between the *midrib* and the two thin, lateral flaps of tissue. An examination of the midrib reveals a large *collateral vascular bundle* in which the phloem is directed towards the *abaxial* or lower leaf surface, whereas the xylem is situated beneath the *adaxial* or upper leaf surface. With this orientation in mind, it will now be clear that the lamina exhibits a typical *dorsiventral character*, shown not only by the relative positions of xylem and phloem in the larger veins but also by the differentiation of the mesophyll into *palisade* and *spongy parenchyma*. Since the anatomy of the lamina in the region of the veins differs somewhat from the interveinal areas, it will be more convenient to describe briefly the various tissues and then to point out their topographical variations. In the lamina of this leaf, three principal tissues are present, viz.:

(*a*) *The epidermis.* The *adaxial epidermis* consists of somewhat oval cells, the outer walls of which are covered by a thin cuticle. Although exact measurements are lacking, there seems to be relatively little difference in the thickness of the inner, outer

[1] Cf. Appendix, p. 216, for technique of clearing.

and radial walls of the epidermis. Observe that many of the epidermal cells possess a protoplast that is peripheral in position. *Stomata* are not uncommon in the adaxial epidermis. Note particularly the relatively small size of the guard cells and the air chamber present beneath each stoma. *Trichomes,* represented by capitate and simple hairs, occasionally develop but these structures are more abundant on the abaxial surface of the lamina. The *abaxial epidermis* is fundamentally similar to the adaxial epidermis except that the cells are somewhat smaller and thinner-walled. Stomata are more abundant in this layer of the lamina, a common situation in many angiosperms. *Multicellular capitate hairs,* lying within shallow depressions, are relatively common and consist of a terminal group of densely protoplasmic *secretory cells* (with rather thick outer walls) seated upon a small unicellular stalk. Haberlandt (1914, p. 240) suggests that the capitate hairs of *Syringa* may absorb thin films of water from the leaf surface but this supposed function requires further investigation.

(*b*) *The mesophyll.* This tissue region is composed of two types of parenchyma, viz.: (1) the *palisade parenchyma,* which is found directly beneath the adaxial epidermis and consists of rather narrow, thin-walled, somewhat "rectangular" cells, the long axes of which are perpendicular to the epidermis. Notice that *intercellular air spaces* are prominently developed in the palisade parenchyma. In addition to a prominent nucleus each palisade parenchyma cell contains a large number of peripheral *chloroplasts.* Directly internal to the abaxial epidermis occurs (2) the *spongy parenchyma,* composed of thin-walled irregular cells that have no definite orientation and are very loosely arranged. Notice that in many instances the "arms" of adjacent cells of the spongy parenchyma touch each other at their narrowest points so that the maximum of wall surface borders upon the large air spaces. The cells contain a peripheral protoplast and a smaller number of chloroplasts than occurs in the cells of the palisade parenchyma. The spongy parenchyma has at least two important *functions.* First, because of its loosely arranged cells, it acts as a "ventilating tissue" of the leaf, i.e., diffusion of CO_2 water vapor and O_2 between the air lucunae and the cells can take

place with relative ease. Second, the spongy parenchyma carries on some photosynthesis, although this function is more efficiently performed by the palisade parenchyma. According to Haberlandt (1914, p. 287) no translocation takes place from one palisade cell to another but "the stream of synthetic products" follows the *long axes* of these elements. Some anatomical evidence in support of this view is furnished by the fact that small groups of 2-10 palisade cells in certain plants converge at their lower ends and rest upon the upper dilated end of a cell of the spongy parenchyma. Haberlandt regards these specialized cells of the spongy parenchyma as *collecting cells* that receive the products of photosynthesis from the palisade cells and transmit them directly or indirectly to the main vascular channels.

(*c*) *The vascular system.* Investigate first the general structure of the midrib, and note particularly the absence of photosynthetic parenchyma from this portion of the blade. Instead of the usual spongy and palisade parenchyma, the median vascular bundle is covered on both sides by a number of layers of "isodiametric" thick-walled cells, the outermost of which are thickened in such a manner as to resemble collenchyma. (Note: *This "replacement" of photosynthetic parenchyma by collenchyma and thick-walled parenchyma occurs to a lesser extent in connection with the smaller vascular bundles of the blade.* In other types of leaves sclerenchyma may be present—mechanically, a "girder effect" is produced.) Several smaller vascular bundles with the phloem toward the adaxial surface are usually seen in the upper part of the midrib; these "*accessory bundles*" unite basally with the single leaf trace. A certain amount of *secondary growth* apparently has taken place in the median vascular bundle, a phenomenon that is of rather general occurrence in the larger veins of dicotyledonous leaves. The conspicuous *secondary xylem* is composed of conducting elements (probably both *tracheids* and *vessels*) and *fibers* arranged in more or less definite radial rows and uniseriate *xylem rays* that extend across the "*cambial zone*" into the *phloem.* The *primary xylem* (which lies above the secondary xylem) consists of cells that show more or less of a radial arrangement and that are embedded among *xylem parenchyma.* The *secondary phloem*

consists of polygonal, thin-walled *sieve-tubes* associated with small, somewhat triangular densely protoplasmic *companion cells*, *phloem parenchyma*, and *uniseriate phloem rays*, each of which frequently terminates in a large parenchyma cell. The *primary phloem* is indefinite and difficult to distinguish. The *cambial zone* can be distinguished but is not nearly as prominent or distinct as in the case of a stem. Nevertheless, three or more radial rows of differentiating cells can be rather clearly seen. In general, cambial activity is never prolonged in the veins of most leaves. In the smaller veins of the lamina, the vascular tissue is considerably reduced in amount, and secondary growth may be entirely lacking. In certain regions of the blade, the diverging bundles may be cut more or less obliquely so that the characteristic type of primary xylem elements may be recognized. The very small *leaf veins* may consist of several parenchyma cells and a few primary xylem elements; a bundle of this character is usually surrounded by a jacket of parenchyma cells containing chloroplasts.

4. *The leaf blade of Zea Mays.* Obtain a transverse section of a corn leaf and examine it under *low power*. The *adaxial epidermis* is readily identified by the presence of groups (3-5 cells) of somewhat lens-shaped, apparently empty cells. These cells are known as *bulliform cells* and by changes in their turgor allow the leaf blade to curl or uncurl, a phenomenon that may be advantageous in restricting the loss of water from the leaf under arid conditions. The typical *epidermal cells* of both the abaxial and adaxial epidermis are somewhat oval in transverse section (actually they are rather elongated cells) and are provided with a definite cuticle. *Stomata,* with conspicuous air-chambers beneath them, are present in *both* epidermal layers. Occasional unicellular, sharp-pointed *hairs* occur on the adaxial epidermis.

The *mesophyll* tissue of the leaf shows no clear differentiation into palisade and spongy parenchyma but instead is composed of several layers of rather compact parenchyma cells.

The *vascular system* of the leaf consists of a parallel series of *collateral bundles*. The majority of the bundles are rather small; at intervals, fairly large bundles occur. Examining one of the *small bundles* under high power, note that it is completely sur-

rounded by a *bundle sheath* of rather large "isodiametric" parenchyma cells that contain large, starch-holding plastids; the bundle sheath may act not only as a conducting layer that presumably transports the products of photosynthesis directly to the phloem, but also as a temporary storage tissue for starch (cf. Rhoades and Carvalho, 1944). The *xylem* of each bundle is directed toward the adaxial surface of the leaf and consists only of small tracheary elements. The *phloem* of the bundle is nearest the abaxial surface of the leaf and at most is formed of a few, small *sieve-tubes* and *companion cells;* in very small bundles, the phloem may be represented by parenchyma cells. The structure of a large *vascular bundle* in the leaf is quite similar to the anatomy of a stem bundle (cf. Exercise XIII, p. 176). Notice particularly the clear distinction between the sieve-tubes and companion cells of the phloem and the presence of an air lacuna at the adaxial edge of the xylem. *Sclerenchyma* is present on both edges of the bundle and may even partially surround it; according to Esau (1943, p. 333 and Fig. 2E), the "sheath cells on the flanks of the bundle are shaped like elongated parenchyma cells and contain abundant chloroplasts." The association of sclerenchyma with the vascular bundle is quite characteristic of grass leaves and is regarded as a very efficient "plan" for securing mechanical strength in the leaf.

5. *The bud scale.* In general, bud scales are distinguished anatomically from foliage leaves by (1) their greatly reduced vascular system, which may consist of a series of parallel or dichotomizing veins, and (2) by a simple type of undifferentiated mesophyll. The outer bud scales of certain trees may produce a well-developed *periderm* beneath the outer epidermis (e.g., *Aesculus*). Mechanical cells, such as *fibers* and *sclereids,* are often prominent, for example in *Camellia, Fagus, Quercus,* and *Populus.* (For further details, consult Foster, 1928, pp. 137-146.) Study prepared transections of the bud scales of several of the forms listed above.

6. *The leaves of gymnosperms.* Examine stained transections of the leaf of *Pinus,* noting the following successive tissues and regions, viz.: (*a*) the thick-walled *epidermis,* with its sunken stomata; (*b*) the subepidermal zone of *sclerenchyma;* (*c*) the

mesophyll, consisting of large chloroplast containing parenchyma cells, with distinctive invaginated flanges protruding into the cell cavities; *resin canals* are conspicuously developed at various regions in the mesophyll; (*d*) *the endodermis,* with well-defined Casparian strips, enclosing (*e*) one or two *collateral vascular bundles* embedded in *transfusion tissue.* The latter consists of cells, which although parenchymatous in form, are provided with circular, bordered pits (for a discussion of the structure and phylogenetic importance of transfusion tissue in the leaves of gymnosperms, cf. Bernard, 1904 and Jeffrey, 1917, Ch. XIV). The late phases of differentiation of the various leaf tissues in *Pinus sylvestris* are described by Tetley (1936).

IV. Suggested Drawings and Notes

1. Prepare drawings to illustrate the initiation and early differentiation of the leaf in (*a*) a grass and (*b*) some species of dicotyledon.

2. Make diagrams to illustrate the arrangement of vascular tissues as observed in the transections of the petioles of the various dicotyledons studied.

3. Show diagrammatically a portion of the venation of the cleared laminae of several leaf types. Draw in detail several vein endings from each type.

4. Prepare a diagrammatic drawing of the entire transection of the lamina of *Syringa,* indicating the position of all important tissues. Make a separate drawing showing the cellular detail of a narrow sector through the thin portion of the lamina; this drawing should include at least one stoma and one or more veinlets.

5. Draw in detail the cellular structure of a sector through the lamina of *Zea.* This drawing should include a group of bulliform cells and at least one well-developed vein.

6. Outline on a large scale the entire transection of the leaf of *Pinus* and fill in the cellular details of small portions of each of the tissues present.

7. Prepare a résumé of the histological aspects of the process of leaf abscission (for discussion and literature cf. Pfeiffer, 1928, and Eames and MacDaniels, 1947, pp. 266-276).

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Exercise XV

THE ROOT

Except in the Psilotales, *Salvinia*, and a few specialized parasitic forms, roots are a typical feature of the sporophyte of vascular plants. In many of the lower tracheophytes, the primary root is short-lived and numerous adventitious roots soon begin development from various portions of the shoot system. In seed plants, however, the radicle often reaches great prominence and in such cases is termed a *tap root*. Additional *fibrous roots* in seed plants arise by successive branchings beginning with the first root but are also commonly formed adventitiously from the stem. Roots perform a number of important physiological functions. Primarily, they serve as organs that absorb water and solutes from the soil solution. In addition, they are very important as structures that anchor the plant firmly in the soil. The storage of reserve food material also occurs in most roots to some extent and is very obviously displayed in the fleshy "roots" of carrot, beet, turnip, and similar economic plants. Although the usual environment of roots is the soil, *aerial roots* are produced in certain vines and epiphytes and serve to attach the shoot firmly to the surface upon which it may be growing.

I. The Primary Structure of the Root

The primary structure of the root in seed plants differs from that of the stem in a variety of ways. In the following résumé, the successive tissues and regions of a root, prior to secondary growth, are briefly discussed. For an exhaustive treatment of root anatomy, the student should consult the recent monographs by von Guttenberg (1940, 1941).

1. *The root apex.* In marked contrast to the *superficial position* of the apical meristem of the shoot, the actual tip of the root consists of the *root cap*, which acts as a protective buffer to the meristem *beneath* it. As explained in Exercise III (p. 37), Hanstein's histogen concept has been applied widely in the interpretation and classification of the so-called "types" of root apices.

But it is evident that the structure and growth of root apices deserve the same kind of searching reinvestigation that has been given in recent years to shoot apices. There is particular need for intensive study of the method of *origin* of the apical meristem of the root *during embryogeny*. Allen (1947) has investigated *Pseudotsuga* from this standpoint and has emphasized not only the *internal origin* of the root meristem (as contrasted with the superficial origin of the shoot apex) but has also described its distinctive *zonal structure* and growth. Similar investigations on other gymnosperms and on a wide variety of angiosperms should aid in clarifying the present confused state of terminology and interpretation with reference to root apices. One of the most essential points deserving emphasis here is that the general activity of a root apex is fundamentally different from that of a shoot apex. The latter gives rise to *exogenous foliar primordia* and to the primary meristematic tissues. In the root, however, two *dissimilar patterns of differentiation* originate from the apical meristem, one leading to the *outward addition* of new cells to the root cap, the other contributing new cells to the body of the root. Lateral roots, in contrast to the exogenous foliar primordia of the shoot, arise *endogenously* from the pericycle, *distal* to the root apex proper. These differences between shoot and root apices are at present impossible to explain, but it is evident that they condition the striking morphological and anatomical differences between root and shoot in the seed plants. (For further treatment of this problem, cf. Priestley and Swingle, 1929; Arber, 1941; and Allen, 1947.)

2. *The epidermis.* The external cell layer of young roots, in a purely topographical sense, represents an *epidermis* and consists of tightly joined cells devoid of a cuticle and of stomata. Distal to the region of cell elongation in the root, many of the young epidermal cells produce tubular unicellular *root hairs*. These structures, which are rarely absent in terrestrial roots, are presumed to function as water and solute absorbing structures. In recent years, the factors determining their occurrence and development have received experimental study (cf. Cormack, 1944, 1945, 1947). In most cases the epidermis is uniseriate and in older portions of a root may die or else become sloughed off as

the result of secondary growth. The aerial roots of many orchids and certain other monocotyledons, however, develop a highly specialized type of multiple epidermis known as the *velamen,* which apparently functions as an absorbing tissue (for details on the ontogeny and structure of the velamen cf. Haberlandt, 1914, pp. 231-235; Engard, 1944; von Guttenberg, 1940, pp. 81-84).

From both an ontogenetic as well as a physiological viewpoint, objections have been raised against the use of the term "epidermis" to designate the surface layer of the root. Von Guttenberg (1940, p. 63), for example, proposes the relatively old term *rhizodermis.* Although a discussion of this problem lies beyond the scope of this book, it is necessary to realize that the well-entrenched assumption that the surface layers of young shoots and roots are homologous has been challenged by recent ontogenetic studies (cf. Allen, 1947).

3. *The cortex.* In the roots of some plants, the cortex is relatively simple in histology, consisting largely of thin-walled lacunate parenchyma tissue. Very frequently, however, especially in the roots of monocotyledons, the outermost region of the cortex consists of one or several layers of compactly arranged living cells with *suberized walls;* these cells collectively represent the *exodermis.* According to von Guttenberg (1943, pp. 2-23), the exodermis, because of its relatively impermeable nature, functions somewhat as does cork in restricting the outward diffusion of water and solutes from the inner tissues of the root. In roots that lack secondary growth (e.g., monocotyledons and certain of the dicotyledons) the cortex is retained throughout life and is provided with various types of "mechanical tissues." *Sclerenchyma,* for example, commonly occurs in many monocotyledonous roots as a cylinder of fibers situated beneath the exodermis. In some cases, *idioblastic sclereids* are profusely developed in the inner region of the cortex (cf. Bloch, 1946). Although it has been asserted that roots lack *collenchyma* (cf. Eames and MacDaniels, 1947, p. 85), this tissue has been reported in the outer region of the cortex of a variety of angiospermous genera (cf. von Guttenberg, 1940, pp. 112-113 and p. 163, Fig. 135).

4. *The endodermis.* With very few exceptions, the roots of

vascular plants are provided with a distinctive uniseriate cylinder of cells termed the "endodermis." This layer, at least in roots, histogenetically represents the inner boundary of the cortex. Because of its great phylogenetic and physiological interest, the endodermis has been studied very intensively and only a very brief résumé of its salient features can be given in this book (for an extensive treatment, cf. von Guttenberg, 1940, 1943).

At the level of maturation of the primary vascular system, the endodermis appears in transectional view as a uniseriate cylinder of living cells, the radial and transverse walls of which are provided with *Casparian strips*. The latter represent continuous bands of chemically modified wall substance that extend along each of the two elongated radial primary walls and across each of the two end-walls of the endodermal cells. When endodermal cells, in this "primary stage of development" are plasmolysed, the protoplasts shrink away from the tangential walls but remain closely attached to the Casparian strips (Bryant 1934; von Guttenberg, 1943, pp. 32-33, Fig. 31). The structural and chemical composition of the Casparian strips in endodermal walls has not yet been entirely clarified, although there is evidence that these bands are composed of both lignin and suberin compounds (cf. van Fleet, 1942). For this reason the endodermis is commonly regarded as physiologically important in controlling the entrance of substances into the vascular system and possibly in preventing the outward "leakage" of water and solutes into the cortex (for discussions of the physiological roles of the endodermis, cf. Priestley and North, 1922; van Fleet, 1942; and von Guttenberg, 1943, pp. 145-156). During the primary phase of development, endodermal cells possess a marked capacity for further growth. This is evident by the extension and division of endodermal cells during the endogenous initiation of lateral roots in the pericycle (cf. Esau, 1940, pp. 192-193). Furthermore, during the early phases of secondary growth in certain roots, the endodermis maintains its continuity by anticlinal cell divisions; the *new walls* resulting from such divisions frequently develop Casparian strips.

In some plants, the endodermis remains in the primary stage and eventually may be sloughed off together with the rest of the

cortex. But in many angiosperms, a more or less continuous suberin-lamella is deposited centripetally upon the primary walls (including the Casparian strip); this stage is termed "secondary" and may be followed by the deposition of additional "tertiary" wall layers. These later developed layers very often are most conspiciously developed on the radial and inner tangential surfaces of the endodermal cells, e.g., in the roots of many monocotyledons (cf. von Guttenberg, 1943, pp. 46-47; Eames and MacDaniels, 1947, p. 160). The so-called *passage cells*, which often occur opposite the xylem strands in thick-walled endodermal cylinders, represent individual endodermal cells that have failed to develop secondary suberin lamellae; such passage cells are presumed to facilitate the entrance of water into the vascular system.

5. *The vascular cylinder.* The outer boundary of the primary vascular system of the root consists of a more or less discrete zone of cells usually termed the *pericycle.* Some authors (e.g., von Guttenberg, 1940, 1943) designate this region by the older term *pericambium.* In most roots, the pericycle is uniseriate and lies in direct contact with the protophloem and protoxylem tissues. The pericycle is defined at an early stage in root histogenesis (in some plants prior to the beginning of vascular differentiation) and retains meristematic potencies to a remarkable degree. From it normally arise (1) the primordia of lateral roots, (2) the phellogen, and (3) portions of the vascular cambium. In the roots of grasses, the outermost protoxylem tracheary elements may differentiate from pericyclic cells. Whether the "pericycle" of roots morphologically represents a tissue region comparable to the so-called "pericycle" in stems is open to question. As Esau (1943c, p. 585) points out, the pericycle of roots needs critical ontogenetic study.

Without doubt, one of the most distinctive features of root anatomy is the method of development and arrangement of the *primary vascular tissues.* In striking contrast to the *collateral* position of primary phloem and primary xylem in the stems of most seed plants, these tissues are arranged in an *alternate and radial* pattern in the root. As seen in transectional view, the primary xylem and primary phloem of the root appear as separate,

alternating strands or plates of tissue separated from each other by parenchyma or sclerenchyma. Very frequently, the two or more xylem plates are joined in the center of the root forming a solid core of xylem. For this reason, the vascular cylinder of roots has often been regarded as a *protostele*. In many roots, however, particularly in the monocotyledons, the center of the vascular cylinder is occupied by a core of parenchyma or sclerenchyma usually designated as the pith. Roots vary widely in respect to the number of primary phloem and primary xylem strands, not only between species or genera but even between the roots of the same individual (cf. Esau, 1941, p. 452, Table I). Depending upon the number of protoxylem groups present, the following descriptive terms are used, viz.: *diarch* (e.g., *Nicotiana, Beta, Daucus, Raphanus*), *triarch* (*Pisum*), *tetrarch* (*Ranunculus, Gossypium*), *pentarch*, etc. The primary vascular cylinder in many monocotyledons consists of a larger number of alternating xylem and phloem strands (100 or more in some of the palms) and hence is designated as *polyarch*. In the roots of certain grasses, a single, large metaxylem vessel extends through the central region of the vascular cylinder (cf. Hayward, 1938, p. 157, Fig. 67).

The *ontogeny* of primary vascular tissues in the roots of seed plants contrasts in two important respects with the situation typical of the stem, viz.:

(*a*) *Radial maturation.* Although the general pattern (i.e., diarch, triarch, etc.) of the future vascular system is more or less completely blocked-out from the surrounding tissue *before* the maturation of sieve-tube and tracheary elements, the actual differentiation of *both* protophloem and protoxylem cells *begins* at the outermost edge of the respective strands (i.e., next to the pericycle) and progresses *centripetally*. For this reason, the primary xylem of roots is termed *exarch* in contrast to the endarch xylem characteristic of the stems of seed plants (cf. Exercise XIII p. 158). In lower vascular plants (i.e., the Psilopsida and Lycopsida), exarch primary xylem occurs in *both* the stem as well as the root. Hence the universal occurrence of exarch xylem in the roots of seed plants might be regarded as the persistence of an ancient type of vascular anatomy (cf. Jeffrey, 1917, Ch. XII).

(*b*) *Longitudinal differentiation.* In contrast to the isolated character of the first formed protoxylem elements in leaf traces, the *protoxylem* of the root (as well as the procambium and protophloem) develops *acropetally* in unbroken continuity with the same tissue in the older portion of the root (cf. Esau, 1943a, p. 165, Fig. 13). Furthermore, in all thoroughly investigated cases, the protophloem sieve-tubes mature nearer the apical meristem of the root than do the youngest tracheary elements.

II. Origin and Development of Lateral Roots

Branching of the stem in seed plants normally originates from axillary buds that, in turn, have developed at or near the shoot apex from superficial cells or cell layers. In marked contrast, the *branching of the root is strictly endogenous.* The origin of lateral root primordia usually occurs by the renewed growth and tangential division of certain groups of pericycle cells distal to the region of elongation of the main root. In some plants, however, such as *Eichornia crassipes,* lateral root primordia arise relatively close to the root apex (Arnold, 1940). In roots with three or more primary xylem strands, lateral root primordia tend to originate from pericycle cells opposite each of the protoxylem poles. Consequently, unless injuries or abnormalities occur, the number of *vertical rows* of lateral roots is equal to the number of xylem strands. But in diarch roots, such as *Daucus,* lateral root primordia arise at each side of the two phloem groups; in this case four rows of lateral roots are developed (Esau, 1940, pp. 190-194).

As growth and cell division continue in the cells of the lateral root primordium, there is gradually differentiated the structure of a typical root tip, including a root cap, apical meristem, cortex, and procambial cylinder. In carrot, "the connection between the vascular tissues of the main and lateral roots is formed through cells of pericyclic origin lying outside the protophloem and protoxylem of the main root" (Esau, 1940). As stated earlier in this exercise, the endodermal cells of the main root may adjust themselves to the outward extension of the lateral root by growth and anticlinal divisions. But in a number of plants, the endodermal cells divide *tangentially* and produce a more or less voluminous

"digestive pouch" over the apex of the emerging primordium. This pouch is short-lived, however, and is eventually destroyed as the lateral root pushes its way through the cortex to the exterior (for detailed discussion and illustrations, cf. von Guttenberg, 1940, pp. 36-40).

III. Secondary Growth in Roots

The roots of many herbaceous dicotyledons and of all gymnosperms and woody dicotyledons exhibit secondary growth. However, because of the radial alternate arrangement of the primary vascular tissues, the *vascular cambium* first appears as separate bands of tangentially dividing cells. Each cambial strip originates from the dividing procambial cells situated at the inner edges of the phloem groups. At these points, the formation of secondary phloem outwardly and of secondary xylem inwardly occurs as in a typical stem. In other words, *collaterally arranged strands of secondary vascular tissues* are built up between the adjacent primary xylem plates. As secondary growth continues, the originally separate strips of cambium finally become united laterally as the result of the tangential division of pericyclic cells external to each protoxylem pole. Thus, during the early phases of secondary growth, the vascular cambium appears *lobed* in transectional view. In many types of roots, those portions of the cambium external to the protoxylem groups function as *ray initials* and build up conspicuous *multiseriate rays*, which are prominent features of the secondary vascular system of many roots (cf. Jeffrey, 1917, pp. 156-157, Figs. 112-113; and Esau, 1943b, p. 315, Fig. 1A). Ultimately, by the more rapid development of tissues between the primary xylem strands, the cambial cylinder becomes circular in transectional view. When secondary growth in a root is pronounced the primary phloem, endodermis, and cortex become crushed and eventually sloughed off. In woody plants, extensive increments of secondary phloem and secondary xylem are produced and, except for the central radial core of exarch primary xylem, all structural resemblance to a root is lost.

The first *periderm layer* in the root is typically produced from a phellogen that arises in the pericycle or its derivative tissues. In *Pyrus*, the pericycle first produces, by repeated tangential

divisions, an extensive zone of tissue, the outer cells of which become suberized before a cork-producing phellogen originates in one of the deeper layers of this zone (Esau, 1943b). As in stems, later formed phellogen layers may develop from the living parenchymatous tissue of the secondary phloem.

IV. Vascular "Transition" Between Root and Stem

One of the most involved problems in plant anatomy has arisen from the numerous attempts to interpret the so-called "transition" from the radial pattern of vascular tissues in the root to the collateral or superposed arrangement of phloem and xylem in the stem of seedlings. Unfortunately, many of the interpretations have been based solely upon the study of serial transections of seedling-axes in which the primary vascular tissues have attained their final development. This emphasis on adult structure has led to the idea that the separate phloem and xylem strands of the root "twist" and become "inverted" in orientation in passing upward through the hypocotyl to the epicotylar axis (Eames and MacDaniels,[1] 1947, pp. 293-296, Fig. 134). According to this viewpoint, the "transition region" may "occur in the top of the radicle, at the very base of the hypocotyl, near its center, or in the upper part. . . . Whenever the inversion of the bundles has not been accomplished at the level at which the cotyledonary traces depart, these outgoing strands are inverted during their passage into the cotyledons." Several recent ontogenetic investigations, however, lend no support to the idea of an *inversion* of strands. In *Daucus*, for example, each cotyledon is supplied by three vascular traces, the origin and structure of which is as follows: The median trace consists of a strand of *exarch xylem, continuous with the protoxylem pole of the root* and flanked laterally by two phloem groups. This strand shows "a centripetal order of differentiation for some distance within the cotyledons." Each of the two lateral cotyledonary traces, in contrast, is collateral in structure, consisting of an outer phloem strand and an inner strand of *endarch xylem.* "The lateral traces show centrifugal xylem differentiation throughout their extent," and originate from the central region of the diarch xylem plate

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in the root. Thus in carrot (and doubtless in other dicotyledons as well) there is strict continuity (without "inversion") between the primary vascular system of the cotyledons and the hypocotyl-radicle region. *After* secondary growth has been initiated in the radicle-hypocotyl region of *Daucus*, the apical meristem of the epicotyl begins to form foliage leaf primordia. Their collateral traces become continuous with the *secondary vascular tissues* of the hypocotyl and radicle. Obviously this type of vascular interconnection between shoot and root is of considerable importance to the concept of "primary" and "secondary" vascular tissues in seed plants and indicates the need for comprehensive ontogenetic studies on a wide range of material (Esau, 1940, p. 204; Miller and Wetmore, 1945, pp. 632-633; Dittmer and Spensley, 1947, pp. 20-23).

V. Material for the Study of the Root

1. For review purposes, study median longisections of the root apices of various angiosperms, noting the method of origin of the root cap and the early phases of development of epidermis, cortex, and vascular cylinder. Also observe demonstrations of transections of young roots illustrating the early stages in maturation of protophloem and protoxylem elements.

2. The primary structure of the root in *Ranunculus* spp. Obtain a prepared transection of the root and study the following tissues and regions, viz.:

(*a*) *The epidermis*, a uniseriate layer of collapsed and partially destroyed cells. A more or less disorganized protoplast may be visible in some of the epidermal cells.

(*b*) *The cortex*, consisting of a narrow outer zone of compactly arranged cells that collectively represent an *exodermis* and a broad inner zone of thin-walled *parenchyma* cells separated from one another by conspicuous intercellular spaces. *Starch grains* are abundant in the cells of the cortical parenchyma.

(*c*) *The endodermis*. Depending upon the level at which the transection was cut, the endodermis will either exhibit only Casparian strips ("primary phase") or a more or less thick secondary deposition of wall material will be noted ("secondary phase").

(*d*) *The vascular cylinder*, the outermost boundary of which is represented by the uniseriate *pericycle*, consists of 3, 4 or 5

radial, alternate strands of primary phloem and primary xylem. Whether this fluctuation in the number of protoxylem and protophloem poles obtains within the different roots of a single species of *Ranunculus* needs clarification. During the centripetal maturation of the primary xylem strands, the cells in the center of the vascular cylinder become metaxylem tracheary elements and a pith is thus absent. Careful study will often reveal the beginning of cambial activity at the inner edge of each phloem strand. According to Maxwell (1893), secondary growth in the root of *Ranunculus* is limited in extent and does not conspicuously affect the original primary structure.

3. *The root of monocotyledons.* Excellent material for a study of the primary structure of a monocotyledonous root is provided by *Smilax herbacea* (for illustrations and discussion of the anatomy of this root, cf. Jeffrey, 1917, pp. 158-159). Obtain a transection cut through the mature region of the root and study the following very clearly defined tissues and regions, viz.:

(*a*) The uniseriate *epidermis,* of tightly joined relatively thick-walled cells.

(*b*) The *cortex,* demarcated from the epidermis by a uniseriate cylindrical layer of cells that constitute the *exodermis.* Occasional thin-walled exodermal cells will be noted but the majority of the elements are provided with conspicuous, unevenly deposited secondary thickenings. The extensive middle zone of the cortex is composed of large, thin-walled parenchyma cells, which contain starch grains and are separated by intercellular spaces. The innermost layer of the cortex is the *endodermis,* a uniseriate cylinder of cells with thick, laminated secondary walls. Careful inspection will reveal *canal-like pits* traversing the much thicker radial and inner tangential portions of these massive secondary wall layers.

(*c*) *The vascular cylinder.* In contrast to the thin-walled and usually uniseriate pericycle of many roots, the peripheral tissue of the vascular cylinder in *Smilax* consists of a zone (5-6 cells in thickness) of thick-walled sclerenchyma. This tissue is interpreted by von Guttenberg (1943, p. 169) as equivalent to the "pericambium" or "pericycle." In common with many monocotyledons, the root of *Smilax* is *polyarch;* in transectional view, twenty or more separate strands of primary xylem alternate with

an equal number of primary phloem groups. These strands are embedded in a thick-walled tissue similar in general appearance to the sclerenchymatous "pericyclic" cylinder. In each phloem strand, the very small *protophloem cells* (lying in contact with the inner edge of the sclerenchyma cylinder) are sharply demarcated from the much larger elements of the *metaphloem.* A comparable difference in size will also be noted between the external *protoxylem cells* and the more deeply situated *metaxylem* elements. The center of the root is occupied by a core of thick-walled parenchyma cells which collectively represent a pith. Starch grains are abundant in these cells.

4. *The origin and development of lateral roots.* Median longisections through the entire terminal portion of the roots of *Pistia* or *Eichhornia* are recommended for a study of the acropetal development of lateral root primordia. In these genera, the abundant lateral roots are initiated relatively near the root apex and a single, thin median longisection will thus provide a closely graded series of stages in lateral root development. Longisections of the primary root of *Zea* should also be studied; they reveal very clearly the earliest phases of initiation and the subsequent development of lateral root primordia from cells of the pericycle. The position of young lateral roots opposite the protoxylem poles of the mother root should also be studied; transections of the root of *Salix* provide very suitable material.

5. *Secondary growth in roots.* Although a wide variety of woody dicotyledons may be used for the laboratory study of this topic, the root of *Pyrus communis* is one of the few species of woody plants in which secondary growth has been investigated in detail. With the aid of the descriptions and abundant illustrations in Esau's (1943b) paper, study a series of successive transections through the pear root, noting (1) the origin of the vascular cambium and the development of secondary vascular tissues, (2) the proliferation of the pericycle and the development of cork, (3) the destruction of the cortex, and (4) the stem-like anatomy of the older region of the root.

VI. Suggested Drawings and Notes

1. Prepare diagrammatic drawings of the transections of the roots of *Ranunculus* and *Smilax* showing the position of all pri-

mary tissues and regions. Draw in detail the cellular structure of a small sector of the inner region of each root showing the endodermis, pericycle, and a portion of the vascular system.

2. Prepare diagrams and detailed drawings, based upon both transverse and longitudinal sections, to illustrate the origin, position, and endogenous development of lateral roots.

3. Prepare a series of transectional diagrams, based on the study of *Pyrus* to illustrate the origin of the cambium and the effects of secondary growth upon the primary structure of the root.

4. Prepare a brief résumé of the methods of origin of "adventitious" roots in seed plants. (For information and literature, cf. Priestley and Swingle, 1929; Hayward, 1938, pp. 53-56; Van der Lek, 1924; Carlson, 1938.)

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APPENDIX

The following brief notes on certain phases of microtechnique are given here to facilitate the use of this book by the teacher and student. For full information on the various procedures used in preparing tissue for microscopic study, reference should be made to the publications of Chamberlain (1932), Rawlins (1933), Johansen (1940), and Sass (1940), cited under "General References."

Free-hand Sections

In many of the exercises in this book, directions are given for the study of sections cut by hand from living stems, leaves or other plant structures. To prepare such material requires only simple technique and in addition provides a realistic picture of cells and tissues that should precede the examination of microtomed and permanently stained preparations. In the laboratory the student can acquire the necessary skill with a sectioning-razor to enable him to explore the structure of such tissues as the epidermis, parenchyma, collenchyma, and the phloem and xylem of vascular bundles. Best results are secured by enclosing small portions of the material between the split halves of pieces of elderberry pith or of carrot root. Often it is necessary to make a groove in the inner surface of one of the pith halves or strips of carrot to accommodate such bulky objects as stems, petioles, etc. In sectioning, hold the object enclosed between the pieces of pith at as constant an angle as possible and transfer each section to water; the slices of pith can be removed by the use of a brush. Sections cut by hand should be carefully mounted on a clean slide either in distilled water or in the various reagents designated and the cover-glass lowered gently into place. For more resistant cells, such as sclereids or fibers and for the critical study of the sieve-plates in phloem elements, the use of the carbon dioxide freezing microtome is highly desirable. With the aid of this instrument a large number of thin sections may be prepared by the instructor in advance of class use (for a description

of the technique of sectioning with the freezing microtome, cf. Sass, 1940, pp. 97-98). The student must learn to check free-hand preparations at frequent intervals so that the sections are not allowed to dry out. Cells immersed in fluid are not only easier to study from an optical point of view, but they also retain a more or less normal structure over a relatively long period of observation. Sections of hairy objects, such as many leaves or stems, are often difficult to mount in water without the formation of numerous air-bubbles. This difficulty may be removed by mounting each section in a weak solution of alcohol. This acts as a killing reagent for the protoplasm, but it does make possible the accurate study of the shape, arrangement, and character of the walls of cells.

Prepared Slides

The use of permanent slides is essential in the study of many of the topics outlined in this book. This is particularly true for the work to be done under Exercises III, X, XII, XIII, XIV, and XV. Suitable preparations as a basis for class study are obtainable from commercial supply houses or may be prepared for the student directly. With reference to the latter possibility, detailed suggestions for the collection, fixation, sectioning, and staining of tissues and organs are presented systematically in the recent manuals on microtechnique by Johansen (1940) and Sass (1940).

Macerated Tissue

One of the most important skills that the student must develop in laboratory practice is the ability to visualize cells as three-dimensional bodies. This is often extremely difficult on the basis of the examination of sections that tend to create a two-dimensional concept. Furthermore, many definitive features of cells, particularly the structure and arrangement of pits and the varied secondary wall thickenings in tracheary elements, the character of perforations in vessel elements and the form of ramified sclereids, can best be studied in isolated cells. For these reasons, a study of macerated tissue is recommended for many topics in this book and is especially desirable in connection with

Exercises II, VIII, IX, and X. The maceration of plant tissue is most effectively accomplished by the use of certain reagents that dissolve the intercellular substance and thus cause the separation of a piece of tissue into its component cells. Jeffrey's method is usually satisfactory, especially for hard lignified structures. Small pieces of the material, no thicker than a match, are placed in a glass vial containing a mixture of equal parts of 10% chromic acid and 10% nitric acid. The vial is then corked and placed in an electric oven at a temperature of 30°-40° C. until the material becomes soft or "mushy" in texture. Hard material, such as wood and the shells of nuts, may require several days in the oven, during which time it is advisable to change the macerating fluid once or twice. Boiling small slivers of wood before placing them in the acids drives out the air and accelerates the maceration process. It is advisable to stop the action of the macerating fluid before a complete separation of all cells has occurred. Small pieces of "mushy" tissue, placed in water on a slide, can be teased apart with needles; such a procedure yields very instructive preparations of connected cell groups as well as isolated elements. The macerated tissue is carefully washed in distilled water to remove as much of the acid as possible and can then be transferred to 50% alcohol for future study. Often effective results may be secured by staining the isolated cells in safranin. Permanent preparations of macerated tissue are easily made by placing small quantities of cells in water on a slide, evaporating the excess water on an electric hot-plate and mounting in glycerine jelly. Circular cover-glasses should be used, the edges of which can be sealed with some type of cement which prevents drying out and the entrance of air.

For some tissues, for example, the cortical and pith parenchyma of herbaceous stems and the mesophyll of leaves, the following more gentle maceration technique should be employed. Place small portions of the tissues in acid-alcohol (3 parts of 70% ethyl alcohol; 1 part concentrated HCl) and thoroughly remove the air by means of an aspirator. Add fresh acid-alcohol and allow this to act on the tissue for 24 hours. After thorough washing in water, transfer the tissue to a 0.5% aqueous solution of ammonium oxalate. Within a few days (or sooner depending

upon the material) the parenchyma or collenchyma tissues can readily be dissociated, by gentle teasing with needles, into their component cells. This method is highly recommended for isolating the idioblastic ramified sclereids of such plants as *Camellia, Osmanthus,* etc., from neighboring tissue elements.

Clearing Technique

It is extremely laborious and in many cases unnecessary to attempt to reconstruct the gross anatomy of the vascular system of a flower or the venation of a leaf by means of serial sections. The following procedure yields very instructive preparations and is applicable to herbarium specimens as well as to fresh material.

1. *Leaves.* Small leaves can be cleared *in toto.* Large laminae, however, should be subdivided into strips (about ¾ inch in width) extending from the mid-rib to the margin. The leaf material of herbarium specimens should first be soaked in hot water until it sinks; with fresh leaves, the chlorophyll should be extracted by means of hot alcohol. Following these preliminary treatments, the material is then transferred to vials containing a 5-10% aqueous solution of NaOH. With delicate objects, the removal of the cell contents may be achieved without heat. But for thick, coriaceous leaves, the vials should be placed in an electric oven. Frequent changes of the reagent are desirable until the leaf material appears devoid of discoloration. After washing thoroughly in distilled water, the material should be examined (without a cover-glass) under low magnification; in many cases the stomata, veinlets, and other histological features (e.g., idioblastic sclereids) can now readily be studied. Cleared material at this stage can be stored for future examination in 50% alcohol. Very often, however, even after several days treatment with NaOH, the material may still be opaque. In such instances, dehydration in successive grades of alcohol followed by clearing with xylene or toluene are necessary. Syracuse watch-glasses are convenient receptacles and the following series is recommended: 50% alcohol, 95% alcohol, 100% alcohol (2 changes), 100% alcohol-xylene, pure xylene. Use a camel-hair's brush or forceps in transferring the material through this series, draining off the excess fluid on filter paper at each change. Be-

cause of the large volume of tissue represented, dehydration must be very thorough or a milky turbidity will form when the final transfer to xylene occurs. To avoid this, allow the material to remain in each of the grades of alcohol for at least 10-15 minutes. After several pieces of leaves have passed through the entire series, the reagents should be discarded and fresh alcohol and xylene added to the watch-glasses. To make permanent mounts, transfer a piece of material to a clean slide, flooded with xylene, add a generous amount of balsam and carefully lower a cover-slip into place. The slides should then be placed on an electric-slide warmer for a week or longer until thoroughly dry. Various types of stains (e.g. safranin, Delafield's hematoxylin) often increase the usefulness of cleared leaf-material. These stains may be introduced at various points in the alcohol-xylene series.

2. *Flowers.* The procedure outlined above is also applicable to the study of the vasculation of the floral axis and its appendages. In making permanent mounts of cleared flowers it is often desirable to use "depression slides"; these permit the inclusion under the cover-slip of relatively thick objects.

Special Reagents

1. Phloroglucinol and hydrochloric acid. The addition of these reagents produces a red color in the walls of sclereids, lignified fibers, and tracheary elements (cf. Exercises VIII, IX and X). The stain is not permanent but nevertheless is extremely useful in demarcating the thick walls of certain types of cells. A saturated solution of phloroglucinol should be prepared in 18% HCl. Mount the section or tissue fragment directly in a drop of this reagent on the slide and add a cover-slip. Great care should be taken to carry out this procedure some distance away from the microscope.

2. Potassium iodide (IKI) and sulphuric acid. This is a specific test for cellulose. Mount the sections in the potassium iodide (1 g. iodine and 3 g. potassium iodide in 300 cc. of distilled water, according to Rawlins, 1933) and add a cover-slip. The introduction of a drop of 65% sulphuric acid will cause cellulose walls to turn blue in color.

3. Anilin blue. This is a specific stain for callus depositions

on sieve plates and is essential for the procedure outlined in Exercise XI. Sections should be immersed for a short time in a .1% aqueous solution, and then transferred, after gentle washing, to a drop of water. The callus on the sieve plates is stained blue. Dr. A. S. Crafts has suggested to the writer the following improvement: Place the sections in IKI, wash in water, stain in anilin blue for about five minutes, then wash briefly again with IKI and mount for study in tap water or glycerine.

4. Neutral red. This vital stain is very useful as a general stain for the primary walls of living cells, and is recommended for use in Exercises V, VI, and VII. Mount the sections directly in a .1% aqueous solution.

5. Sudan IV. This reagent is specific for the cuticle and for cutinized and suberized cell walls. Place the sections in a drop of alcoholic solution of Sudan IV (.5 g. in 100 cc. of 80% alcohol), add a cover-glass, and examine under the microscope. The cuticle, as well as waxy materials present in walls, are stained red. This reagent is very desirable for use with Exercise V.

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